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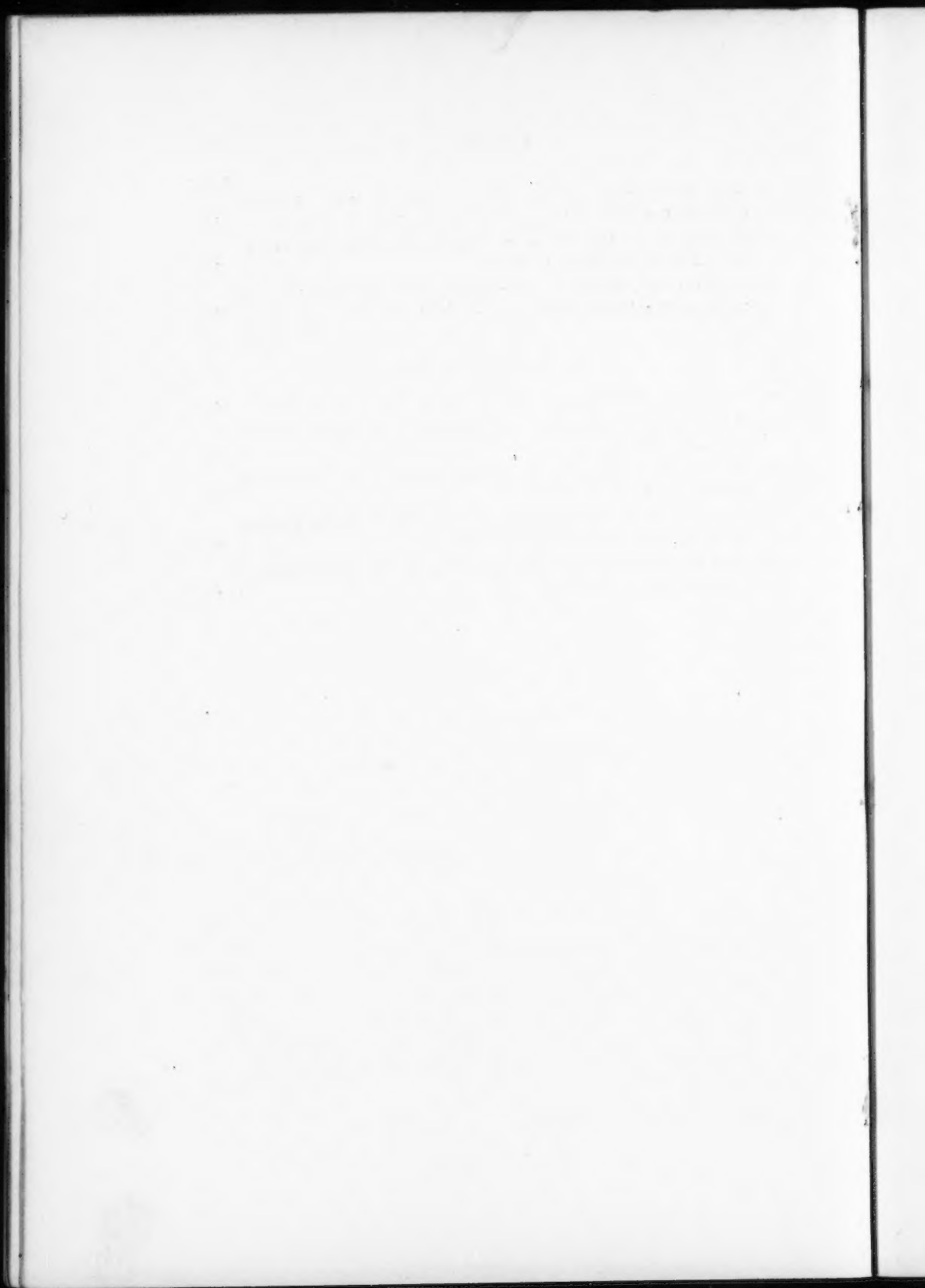
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THE
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SEPTEMBER 1, 1904.

NO. I.

ON THE COMBINED ACTION OF PROTEOLYTIC
ENZYMES.¹

BY P. A. LEVENE AND L. B. STOOKEY.

[From the Department of Physiological Chemistry of the Pathological Institute of the
New York State Hospitals.]

THE question of interaction of enzymes of different origin has attracted considerable attention during recent years. The observation of Cohnheim² on the action of pancreatic extracts upon the glycolytic power of other organs was the most important in that direction. Similar observations were made in the laboratory of Salkowski by Arnheim and Rosenbaum.³

In May, 1903, at the meeting of the American Association of Pathologists and Bacteriologists, we communicated some results of our investigations "On the Digestion and Self-Digestion of Tissues and Tissue-Extracts."⁴ It was noted that the proteolytic action of fresh extracts of the liver and of the spleen is more powerful than the action of one of them on the heated extract of the other. Theoretically there may be several explanations; the higher activity under the conditions of our first experiments might be ascribed to the greater concentration of the proteolytic enzymes, or to the action of the extract of one organ on the zymogen of the other.

Halpern⁵ in Salkowski's laboratory made some observations very

¹ Read at the annual meeting of the Association of American Pathologists and Bacteriologists, April 1, 1904.

² COHNHEIM: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 337.

³ ARNHEIM and ROSENBAUM: *Ibid.*, 1903, xl, p. 220.

⁴ LEVENE and STOOKEY: *Journal of medical research*, 1903, x, p. 217.

⁵ HALPERN: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 377.

similar to ours. His object was the study of the action of the extract of the liver on the proteolytic power of the pancreas. Halpern's conclusion was that the liver may aid the pancreas in its proteolytic action. The conditions of the experiments, however, were such that no decisive conclusion could be reached.

Previous to these experiments, this subject attracted considerable attention in connection with the question of the influence of the spleen on pancreatic secretion. A critical review on the subject was given by Mendel and Rettger.¹ Most of their experiments had been done previous to the discovery of proteolytic enzymes in animal organs, and were discontinued after the presence of such enzymes had been demonstrated in all animal tissues.

The present paper represents the results of observations made on the combined action of spleen and pancreas, and of spleen and liver. Extracts of the organs were used in some of the experiments, and minced glands were employed in the latter part of the investigation. It was found that when spleen and pancreas were allowed to act simultaneously on a foreign proteid,—egg albumin or casein,—the quantity of products of digestion resulting from their action was greater than the total sum of products obtained by the digestion of equal quantities of spleen and pancreas acting separately. It was thus not a summation of the action of two enzymes, but an increase in the digesting energy of one or both. However, from the results of some of the experiments the increased action might be attributed to the pancreas. Thus when three parts of pancreas and one part of spleen were allowed to act on a foreign proteid, the quantity of digestion-products was greater than in the experiments where pancreas and spleen were taken in equal proportions or in proportion of one part of pancreas to three of spleen. Another experiment seems to add some weight to this supposition. Fresh pancreas was allowed to stand under antiseptic precautions for about forty-eight hours at room-temperature. At the end of that time, all the zymogen of the gland presumably is transformed into enzyme. Fresh spleen was added, and their combined action on foreign proteid was not noticeably greater than an equal portion of pancreas similarly treated and an equal quantity of spleen acting separately. Thus, these experiments seem to corroborate the views of Schiff, Herzen, and Mendel and Rettger, namely, that the spleen facilitates the transformation of the pancreatic zymogen into the active enzyme.

¹ MENDEL and RETTGER: This journal, 1902, vii, p. 387.

It was impossible to detect a similar action on the part of the spleen upon the proteolytic power of the liver. The products of digestion obtained by the combined action of the liver and of the spleen were not greater in quantity than those obtained by the action of the spleen and the liver separately.

EXPERIMENTAL PART.

Extracts. — Fresh organs were freed from adhering tissues, minced, taken up in 0.25 per cent of sodium carbonate solution (pancreas), or in physiological salt solution (liver, spleen), and allowed to stand over night in a refrigerator. Chloroform and toluol were used as antiseptics in all experiments. At the end of that time, the extracts were filtered, also in a refrigerator, and the filtrates obtained in this manner were used for the experiments.

In the other experiments, fresh glands were minced, taken up in 0.25 per cent sodium carbonate, or in physiological salt solution, and thus were used for the investigations.

In order to estimate the quantity of products of digestion, a part of the mixture was saturated with zinc sulphate, and another part was treated with phosphotungstic acid. In the filtrates nitrogen determinations were made. The zinc sulphate filtrate was prepared in the following manner: The mixture was made acid, saturated with zinc sulphate, and brought to a definite volume by means of a saturated solution of zinc sulphate. It was then allowed to stand over night, filtered, and an aliquot part of the filtrate was used for analysis. The phosphotungstic filtrate was prepared in an analogous manner.

Each experiment consisted of the principal one, in which the two enzymes were allowed to act simultaneously, and of controls in which the proteid was subjected to the action of only one gland or extract.

The results of our experiments are given in the following tables:

TABLE I.

No. OF EXP.	SUBSTANCES.	QUAN- TITY.	VOL. OF SAL- INE.	BEFORE DIGESTION.				LENGTH OF DIGES- TION IN HOURS.	AFTER DIGESTION.			
				N in Zn filtrate.		N in W filtrate.			N in		N of foreign protein in	
				gram	per cent	gram	per cent		Zn filtrate. grams	W filtrate. grams	Zn filtrate. gram	W filtrate. gram
I a	Saline extract of spleen	..	c.c. 50	0.77	32.3	0.50	21.0	24	0.92	0.59		
b	{ Saline extract of spleen { Egg albumin	2.5	50 50	0.86	15.6	0.62	11.1	24	1.04	0.76	0.03	0.05
c	Saline extract of pancreas	..	50	0.76	66.2	0.44	38.5	24	0.87	0.51		
d	{ Saline extract of pancreas { Egg albumin	2.5	50 50	0.92	..	0.72	..	24	1.28	1.06	0.25	0.27
e	{ Saline extract of pancreas { Saline extract of spleen { Egg albumin 2.5	25 25 50	0.41	..	0.36	..	24	1.39	1.08	0.65	0.61
II a	Spleen	5.0	30	0.016	10.5	0.005	3.6	24	0.029	0.02		
b	Pancreas	10.0	60	0.023	8.5	0.012	4.5	24	0.11	0.08		
c	{ Spleen { Sodium caseinogenate	10.0 2.0	80	0.036	..	0.01	..	24	0.15	0.09	0.09	0.04
d	{ Spleen { Sodium caseinogenate	15.0 3.0	120	0.048	..	0.015	..	24	0.22	0.13	0.13	0.07
e	{ Pancreas { Sodium caseinogenate	10.0 2.0	80	0.023	..	0.012	..	24	0.69	0.39	0.58	0.31

<i>f</i>	{ Pancreas Spleen Sodium caseinogenate }	10.0 10.0 4.0	160	0.055	..	0.022	..	24	1.45	0.90	0.69	0.46
<i>g</i>	{ Pancreas Spleen Sodium caseinogenate }	5.0 15.0 4.0	160	0.05	..	0.02	..	24	1.20	0.62	0.76	0.36
III <i>a</i>	Spleen	20.0	160	0.06	12.0	0.04	7.8	24	0.08	0.05		
<i>b</i>	Pancreas	20.0	120	0.05	10.4	0.015	3.2	24	0.42	0.089		
<i>c</i>	{ Pancreas Sodium caseinogenate }	20.0 4.0	160	0.05	..	0.015	..	24	1.38	0.77	0.95	0.68
<i>d</i>	{ Pancreas Sodium caseinogenate }	10.0 2.0	80	0.02	..	0.008	..	24	0.71	0.36	0.49	0.32
<i>e</i>	{ Spleen Sodium caseinogenate }	20.0 4.0	160	0.06	..	0.04	..	24	0.14	0.09	0.07	0.04
<i>f</i>	{ Spleen Sodium caseinogenate }	10.0 2.0	80	0.03	..	0.02	..	24	0.07	0.05	0.04	0.02
<i>g</i>	{ Spleen Pancreas Sodium caseinogenate }	10.0 10.0 4.0	160	0.06	..	0.04	..	24	1.65	0.89	1.40	0.82
<i>h</i>	{ Spleen Pancreas Sodium caseinogenate }	20.0 10.0 6.0	240	0.09	..	0.06	..	24	1.98	1.08	1.70	0.98
<i>i</i>	{ Spleen Pancreas Sodium caseinogenate }	10.0 20.0 6.0	240	0.09	..	0.06	..	24	2.23	1.19	1.77	1.08

TABLE II.

No. OF EXP.	SUBSTANCES.	QUAN- TITY.	VOL.	BEFORE DIGESTION.				LENGTH OF DIGES- TION IN HOURS	AFTER DIGESTION.			
				N in Zn filtrate.		N in W filtrate.			N in		N of foreign protein in	
				T. N.		T. N.			Zn filtrate.		Zn filtrate.	
				gram	per cent	gram	per cent		gram	gram	gram	gram
I a	Saline extract of spleen	grams ..	c.c. 100	0.088	23.3	0.069	18.3	24	0.178	0.128		
b	0.25 % Na ₂ CO ₃ extract of pancreas	..	100	0.242	64.0	0.156	41.3	24	0.341	0.222		
F. c	{ 0.25 % Na ₂ CO ₃ extract of pancreas { Egg albumin 5	100 100	0.242	..	0.18	..	24	0.734	0.386	0.393	0.164
d	{ Saline extract of spleen { Egg albumin 5	100 100	0.91	..	0.82	..	24	0.148	0.112	None	None.
e	{ Saline extract of spleen { 0.25 % Na ₂ CO ₃ extract of pancreas { Egg albumin	50 50 100	0.184	..	0.15	..	24	0.626	0.446	0.317	0.221
f	{ Saline extract of spleen { 0.25 % extract of pancreas { Egg albumin 5	80 20 100	0.133	..	0.11	..	24	0.430	0.304	0.220	0.169
g	{ Saline extract of spleen { 0.25 % extract of pancreas { Egg albumin 5	20 80 100	0.234	..	0.18	..	24	0.664	0.338	0.250	0.279
II a	0.5 % Na ₂ CO ₃ extract of pancreas.	..	100	0.44	66.6	0.203	30.4	24	0.592	0.332		
b	Saline extract of spleen	100	0.096	25.4	0.067	17.9	24	0.137	0.095		
c	{ 0.5 % Na ₂ CO ₃ extract of pancreas. { Egg albumin 5	100 100	0.48	..	0.25	..	24	0.994	0.626	0.402	0.294

<i>d</i>	{ Saline extract of spleen Egg albumin	5	100	0.114	..	0.096	..	24	0.156	0.118	0.019	0.023
<i>e</i>	{ 0.5% Na ₂ CO ₃ extract of pancreas Saline extract of spleen Egg albumin	5	50 50 100	0.26	..	0.17	..	24	0.778	0.532	0.414	0.319
<i>f</i>	{ 0.5% Na ₂ CO ₃ extract of pancreas Saline extract of spleen Egg albumin	5	20 80 100	0.18	..	0.10	..	24	0.478	0.378	0.251	0.236
<i>g</i>	{ 0.5% Na ₂ CO ₃ extract of pancreas Saline extract of spleen Egg albumin	5	80 20 100	0.36	..	0.22	..	24	0.920	0.532	0.420	0.248
III <i>a</i>	Saline extract of spleen	100	0.097	20.3	0.074	15.4	24	0.152	0.094		
<i>b</i>	0.25% Na ₂ CO ₃ extract of pancreas	100	0.32	70.0	0.18	38.9	24	0.38	0.24		
<i>c</i>	{ Saline extract of spleen Egg albumin	5	100 100	0.11	..	0.09	..	24	0.296	0.152	0.144	0.059
<i>d</i>	{ 0.25% Na ₂ CO ₃ extract of pancreas Egg albumin	5	100 100	0.33	..	0.21	..	24	0.74	0.43	0.36	0.20
<i>e</i>	{ 0.25% Na ₂ CO ₃ extract of pancreas Egg albumin	5	20 100	0.07	..	0.05	..	24	0.144	0.096	0.068	0.046
<i>f</i>	{ 0.25% Na ₂ CO ₃ extract of pancreas Saline extract of spleen Egg albumin	5	50 50 100	0.26	..	0.15	..	24	0.56	0.33	0.30	0.16
<i>g</i>	{ 0.25% Na ₂ CO ₃ extract of pancreas Saline extract of spleen Egg albumin	5	80 20 100	0.30	..	0.20	..	24	0.61	0.35	0.28	0.14
<i>h</i>	{ 0.25% Na ₂ CO ₃ extract of pancreas Saline extract of spleen Egg albumin	5	20 80 100	0.17	..	0.13	..	24	0.32	0.20	0.12	0.07

TABLE II — continued.

No. OF EXP.	SUBSTANCES.	QUAN- TITY.	VOL.	BEFORE DIGESTION.				LENGTH OF DIGES- TION IN HOURS.	AFTER DIGESTION.			
				N in Zn filtrate.		N in W filtrate.			N in		N of foreign proteid in	
				gram	per cent	gram	per cent		Zn filtrate.	W filtrate	Zn filtrate.	W filtrate.
IV <i>a</i>	Spleen	gram 10	c.c. 40	0.020	6.7	0.014	4.7	24	gram 0.055	gram 0.051	gram	gram
<i>b</i>	Pancreas	10	40	48	0.126	0.040		
<i>c</i>	{ Pancreas after 48 hours at 40° Sodium caseinogenate	10 5	40 100	0.126	..	0.040	..	24	0.578	0.268	0.452	0.228
<i>d</i>	{ Pancreas after 48 hours at 40° Spleen Sodium caseinogenate	10 10 5	40 100	0.146	..	0.054	..	24	0.725	0.352	0.484	0.241
<i>e</i>	{ Spleen Sodium caseinogenate	10 5	.. 100	0.02	..	0.014	..	24	0.095	0.070	0.04	0.02

TABLE III.

No. OF EXP.	SUBSTANCES.	QUAN- TITY.	VOL. OF SAL- INE.	BEFORE DIGESTION.				LENGTH OF DIGES- TION IN HOURS.	AFTER DIGESTION.			
				N in Zn filtrate.		N in W filtrate.			N in		N of foreign protein in	
				gram	per cent	gram	per cent		Zn filtrate.	W filtrate.	Zn filtrate.	W filtrate.
<i>1 a</i>	Spleen	grams 10	c.c. 60	0.024	8.0	0.009	3.0	24	0.051	0.036	gram	gram
<i>b</i>	Liver	10	60	0.024	7.9	0.02	6.5	24	0.053	0.033		
<i>c</i>	{ Spleen { Sodium caseinogenate	5 1	40	0.012	..	0.005	..	24	0.050	0.028	0.024	0.009
<i>d</i>	{ Spleen { Sodium caseinogenate	15 3	120	0.036	..	0.027	..	24	0.155	0.077	0.077	0.021
<i>e</i>	{ Liver { Sodium caseinogenate	5 1	40	0.012	..	0.010	..	24	0.068	0.036	0.042	0.019
<i>f</i>	{ Liver { Sodium caseinogenate	15 3	120	0.036	..	0.030	..	24	0.213	0.110	0.143	0.060
<i>g</i>	{ Liver { Spleen { Sodium caseinogenate	10 10 4	160	0.048	..	0.029	..	24	0.288	0.151	0.173	0.080
<i>h</i>	{ Liver { Spleen { Sodium caseinogenate	15 5 4	160	0.048	..	0.028	..	24	0.257	0.135	0.151	0.060
<i>i</i>	{ Liver { Spleen { Sodium caseinogenate	5 15 4	160	0.048	..	0.034	..	24	0.250	0.128	0.146	0.056

TABLE III — *continued.*

No. OF EXP.	SUBSTANCES.	QUAN- TITY.	VOL. OF SAL- INE.	BEFORE DIGESTION.				LENGTH OF DIGES- TION IN HOURS.	AFTER DIGESTION.			
				N in Zn filtrate.		N in W filtrate.			N in		N of foreign protein in	
				gram	per cent	gram	per cent		Zn filtrate.	W filtrate.	Zn filtrate.	W filtrate.
II <i>a</i>	Spleen	5	c.c. 30	0.010	6.9	0.008	5.4	24	0.020	0.012	gram	gram
<i>b</i>	Liver	5	30	0.010	7.1	0.009	6.4	24	0.022	0.012		
<i>c</i>	{ Spleen { Sodium caseinogenate	5 3	120	0.010	..	0.008	..	24	0.032	0.016	0.012	0.003
<i>d</i>	{ Spleen { Sodium caseinogenate	15 3	120	0.030	..	0.024	..	24	0.070	0.044	0.010	0.006
<i>e</i>	{ Liver { Sodium caseinogenate	5 3	120	0.010	..	0.009	..	24	0.038	0.015	0.016	0.002
<i>f</i>	{ Liver { Sodium caseinogenate	15 3	120	0.030	..	0.027	..	24	0.065	0.037	None	None.
<i>g</i>	{ Liver { Spleen { Sodium caseinogenate	5 5 3	120	0.020	..	0.018	..	24	0.032	0.022	None	None.
<i>h</i>	{ Liver { Spleen { Sodium caseinogenate	15 5 3	120	0.040	..	0.034	..	24	0.110	0.057	0.023	0.008
<i>i</i>	{ Liver { Spleen { Sodium caseinogenate	5 15 3	120	0.040	..	0.033	..	24	0.065	0.040	None	None.

<i>J</i>	Liver Spleen Sodium caseinogenate	15 15 3	120	0.060	..	0.051	..	24	0.110	0.063	None	None.
III <i>a</i>	Spleen	5	30	0.010	6.9	0.008	5.4	48	0.022	0.017		
<i>b</i>	Liver	5	30	0.010	7.1	0.009	6.4	48	0.024	0.017		
<i>c</i>	Spleen Sodium caseinogenate	5 3	120	0.010	..	0.008	..	48	0.084	0.040	0.061	0.024
<i>d</i>	Spleen Sodium caseinogenate	15 3	120	0.030	..	0.024	..	48	0.126	0.065	0.060	0.014
<i>e</i>	Liver Sodium caseinogenate	5 3	120	0.010	..	0.009	..	48	0.094	0.047	0.069	0.030
<i>f</i>	Liver Sodium caseinogenate	15 3	120	0.030	..	0.027	..	48	0.122	0.057	0.048	0.006
<i>g</i>	Liver Spleen Sodium caseinogenate	5 5 3	120	0.020	..	0.017	..	48	0.089	0.045	None	None.
<i>h</i>	Liver Spleen Sodium caseinogenate	15 5 3	120	0.040	..	0.035	..	48	0.131	0.068	None	None.
<i>i</i>	Spleen Liver Sodium caseinogenate	15 5 3	120	0.040	..	0.033	..	48	0.126	0.070	None	None.
<i>j</i>	Spleen Liver Sodium caseinogenate	15 15 3	120	0.060	..	0.051	..	48	0.141	0.075	None	None.

Table I shows the action of pancreas and spleen on foreign proteid.

Table II shows the action of spleen and pancreas in which the zymogen has been transformed into enzyme.

Table III shows the combined action of liver and spleen on foreign proteid.

The authors wish to acknowledge their indebtedness to Professor R. H. Chittenden, under whose control this investigation was made.

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THE EFFECT OF ALCOHOL AND ALCOHOLIC FLUIDS UPON THE EXCRETION OF URIC ACID IN MAN.

By S. P. BEEBE.

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MANY experiments have been made to determine the fate of alcohol in the body. It was believed until comparatively recent time that alcohol was not oxidized in the body, but was excreted, unchanged, by the lungs, skin, and kidneys. This view was held because no specific oxidation products could be found in the excretions. The same objection applies to sugar, which is undoubtedly oxidized in the body. Recent investigations¹ have shown that when the daily dose of alcohol does not exceed 72.5 gms., not more than two per cent leaves the body unchanged, the remainder being oxidized into carbon dioxide and water.

The faulty methods and technique of the older experiments led to widely divergent results and many disputes regarding the food-value of alcohol. Within the last five years, a series of remarkably accurate and careful experiments by men of opposite opinions has led to the following concordant results:

First, alcohol may take the place of some fat or carbohydrate in the food.

Second, when taken to replace isodynamically a given amount of fat in a body unaccustomed to its use, it causes for a few days, from three to six, an increased excretion of nitrogen. This may be due to what Neumann² calls its action as a protoplasmic poison. Following this, there is a proteid-sparing effect practically equal to that of the fat that has been replaced.

Third, alcohol is an extraordinary food, to be used only in certain conditions, as in sickness, when its ease of absorption and oxidation may be of great benefit; but on account of its peculiar toxic effects, it should not be taken except when needed.

¹ BENEDICT: Boston medical and surgical journal, 1902, pp. 31-34.

² NEUMANN: Archiv für Hygiene, 1899, xxxvi, p. 1.

In the experiments from which these conclusions were drawn, the total nitrogen in the urine was the most important factor determined. This gives a measure of the proteid metabolism in the body, but it affords no clue to the distribution of this nitrogen among the several constituents of the urine.

It is now well known that impairment of the functions of certain organs results in the appearance in the urine of nitrogenous compounds which do not normally occur there. In certain diseases of the liver, acute yellow atrophy, or carcinoma, the same quantity of nitrogen may be excreted as in health, but a portion of it is in the form of the amino acids, leucin and tyrosin, compounds never found in healthy urine. It seems probable from the results of recent work¹ that this impairment in function may show itself in an abnormal distribution of the nitrogen among the several physiological constituents of the urine. May not, therefore, alcohol show its toxic effect by impairing the metabolic functions of certain organs to such an extent as to show itself in the forms by which this total amount is excreted.

The various factors which influence the production and excretion of uric acid have been so thoroughly discussed in some of the recent reviews² that it is necessary to refer here only to those experiments in which alcohol has been used.

Some of these, made by methods which have little standing in scientific work, will not be quoted. One of the earliest experiments was that of Chittenden,³ who, in studying the effect of alcohol upon the nitrogenous metabolism of dogs, found that during the alcohol period the uric acid was increased nearly fifty per cent, while the total nitrogen was practically unchanged. The animals received large doses of alcohol.

Herter and Smith⁴ tried experiments on men. In one case whiskey in increasing doses for three days, — 2 ounces, $3\frac{1}{2}$ ounces, and 6 ounces, — was taken, but no appreciable effect could be noticed. With champagne containing an equivalent amount of alcohol, the habitual ratio between urea and uric acid was disturbed, owing to a slight in-

¹ VON JAKSCH: *Zeitschrift für physiologische Chemie*, 1903, xl, p. 123. *Zeitschrift für klinische Medizin*, 1904, xl, p. 249.

² *Ergebnisse der Physiologie*, 1902, I, *Biochemie*, pp. 555-650; 2, *Ibid.*, pp. 377-432.

³ CHITTENDEN: *Journal of physiology*, 1891, xii, p. 220.

⁴ HERTER and SMITH: *New York medical journal*, 1892, p. 617.

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crease of uric acid. The champagne experiment was for one day only. In both of these experiments, some care was exercised as to the diet, but the important influence of nuclein food was not then appreciated, and the ratio of urea to uric acid was the factor most sought for.

The experiments of Donogany¹ on dogs, in which he gave alcohol for one day only, and observed the change in the uric acid excretion, are of little value because he used an obsolete method of analysis. His results follow :

A. 9 c.c. absolute alcohol, 1.1 c.c. per kilo, 0.17 — 0.22 to 0.32 uric acid.

B. 15 c.c. absolute alcohol, 1.5 c.c. per kilo, 0.12 — 0.33 to 0.40.

C. 20 c.c. absolute alcohol, 2.0 c.c. per kilo, no change.

The best experiments thus far recorded are those by Hans Haeser² under Rosemann's directions. These were tried on a young man in good health, unaccustomed to the use of alcohol, the diet was uniform and the methods of analysis were satisfactory.

a. First there was a 10 day normal period for control.

b. Second, a period of 10 days when from one to two litres of water were added to the previous diet.

c. Third, a 10 day alcohol period in which 75 c.c. of 96 per cent alcohol diluted with 1500 c.c. water were taken.

Normal, 0.8288 gms. uric acid per day	} average 0.7732
Water, 0.5879 " " " " "	
Alcohol, 0.7844 " " " " "	

Comparing the alcohol period with the average for the fore period there is no increase, and Rosemann believes that in health a moderate amount of alcohol has no effect on uric acid excretion.

All these experiments are unsatisfactory in many ways, and, at the suggestion of Professor Chittenden, the writer has endeavored to determine the effect of alcoholic fluids upon the excretion of uric acid in man.

METHODS OF ANALYSIS.

Total nitrogen was estimated by the Kjeldahl method; urea, by the Mörner-Sjöquist method; ammonia, by Folin's³ earlier method, and uric acid, by the Hopkins-Folin method.⁴

¹ DONOGANY und TIBALD: *Ungarisches Archiv für Medizin*, 1894, iii, p. 189.

² ROSEMAN: *Deutsche medizinische Wochenschrift*, 1901, p. 213.

³ FOLIN: *Zeitschrift für physiologische Chemie*, 1901, xxxii, p. 515.

⁴ FOLIN: *Zeitschrift für physiologische Chemie*, 1901, xxxii, p. 567.

The first two have been accepted methods for years, and need no comment. The third is admitted by its author to be slightly inaccurate on theoretical grounds, but the writer is of the opinion that the author's reasons are not sufficient to condemn the method, and actual experiment has shown the results obtained by it to be accurate to within five per cent. The uric acid method is accurate to within five per cent, and has been used by a number of investigators.

EXPERIMENTAL SUBJECTS.

Most of the experiments were made on the same person, the writer, a young man in good health, of regular habits, unaccustomed to the use of alcohol in any form. Some of the later experiments were made upon other students about the laboratory, but none of the subjects was in the habit of using alcoholic fluids. The subjects will be designated by letter, A, B, C, D, E.

Experiment 1.—In Experiment 1 absolute alcohol suitably diluted with water was used. The experiment lasted sixteen days, the first seven being a control, followed by a six-day alcohol period, and this in turn by a four-day after period. The habits of the subject (A) were regular, there being no unusual work or exertion at any time during the experiment. The alcohol was taken at various times during the experimental day, which began at 7 A. M. It was not sufficient in amount to cause any annoyance except on the last day, when 90 c.c. were taken.

The diet given below was uniform throughout; the same quantity and quality of food used each day of the experiment.

Breakfast.	Lunch.	Supper.
200 c.c. milk.	500 c.c. milk.	90 gms. cooked potato.
35 gms. "Force."	50 gms. dried beef.	100 gms. beefsteak.
50 gms. sugar.	100 gms. "Uneeda" biscuit.	60 gms. bread.
300 c.c. coffee.	40 gms. sauce.	30 gms. butter.
60 gms. bread.		300 c.c. water.
30 gms. butter.		

A summary of the analyses is given in Table I.

Effect of Alcohol upon the Excretion of Uric Acid. 17

TABLE I.
SUMMARY OF EXPERIMENT I.

Date.	Volume.	Nitrogen.	Ammonia	Urea.	Uric acid.	Alcohol.
	c.c.	GRAMS	GRAM	GRAMS	GRAM	c.c.
Oct. 12	1310	11.92	0.347	0.540	
" 13	1310	11.67	0.472	9.55	0.562	
" 14	795	13.01	0.616	11.88	0.601	
" 15	1000	15.59	0.681	13.72	0.695	
" 16	1000	14.17	0.736	12.45	0.624	
" 17	820	12.59	0.764	10.65	0.652	
" 18	1010	12.37	0.574	10.86	0.675	
" 19	880	13.72	0.653	11.61	0.618	50
" 20	1120	13.09	0.755	11.40	0.663	60
" 21	1320	14.04	0.725	12.24	0.798	70
" 22	1300	12.66	0.615	11.28	0.820	70
" 23	1200	13.50	0.625	12.14	0.787	80
" 24	1600	14.20	0.695	12.96	0.648	90
" 25	1400	12.66	0.590	10.60	0.600	
" 26	1050	13.50	0.535	11.93	0.634	
" 27	930	13.71	0.455	12.56	0.647	
" 28	1130	14.76	0.600	13.30	0.579	
DAILY AVERAGES.						
		Ammonia.	Total nitrogen.	Uric acid.		
		GRAM	GRAMS	GRAM		
Fore period		0.640	13.25	0.635		
Alcohol period		0.678	13.46	0.755		
After period		0.542	13.65	0.615		

The effect of the alcohol was to cause an increase in the uric acid excretion. This increase as taken from the daily averages amounted to 18.8 per cent of the amount excreted daily during the fore period. It must be noted, however, that the nitrogen and uric acid of the first

TABLE II.
SUMMARY OF EXPERIMENT 2a.

Date.	Volume.	Nitrogen.	Ammonia.	Urea.	Uric acid.	Whiskey.
	c.c.	GRAMS	GRAMS	per cent	GRAM	c.c.
Nov. 20	1200	13.32	0.632	..	0.551	
" 21	1300	15.63	0.624	91.1	0.562	
" 22	1600	15.51	0.721	89.6	0.545	
" 23	1350	15.19	0.795	88.3	0.523	
" 24	1050	14.36	0.653	89.7	0.534	
" 25	1230	16.89	0.945	91.9	0.595	
" 26	1200	16.23	0.708	90.4	0.551	
" 27	1595	16.89	1.082	86.8	0.552	120
" 28	1180	15.60	0.848	91.0	0.600	150
" 29	1240	15.93	0.848	87.0	0.628	150
" 30	1310	15.60	0.889	90.7	0.584	150
Dec. 1	1300	14.40	0.834	87.3	0.606	150
" 2	1320	15.93	0.740	87.5	0.656	150
" 3	1320	16.56	0.665	92.0	0.595	150
" 4	1310	15.42	0.625	91.2	0.557	
" 5	1200	16.26	0.722	92.0	0.612	
" 6	1320	18.45	0.830	89.9	0.573	
" 7	1000	15.63	0.740	90.6	0.562	
" 8	1250	16.89	0.720	88.8	0.556	
Dec. 9	1200	15.84	0.903	90.7	0.628	160
" 10	1100	16.26	0.834	89.5	0.628	160
DAILY AVERAGES.						
		Nitrogen.	Ammonia.	Uric acid.	Urea.	
		GRAMS	GRAM	GRAM	per cent	
Fore period		15.01	0.741	0.554	89.6	
Alcohol period		15.73	0.873	0.604	87.8	
After period		16.53	0.717	0.572	90.8	
High ball period . .		16.05	0.868	0.628	90.1	

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two days of the fore period were much below the average. This is due to the fact that the subject was not in the habit of using so much meat as the experimental diet contained. If these two days are excluded, as they properly should be, the increase is 16 per cent, an amount which is sufficient to have some significance.

Experiment 2 a. — The second experiment was made upon the same subject (A) as Experiment 1, the chief difference being that the alcohol was taken in the form of whiskey containing 45 per cent alcohol. The diet contained more proteid but less purin constituents, eggs being substituted for meat.

Again there appears (Table II) a small but undoubted increase in the uric acid output. The increase, 9 per cent, is smaller than was obtained in Experiment 1, but the volume of whiskey taken did not contain so much alcohol as was used in the previous case. The nitrogen intake was larger, but there was less purin material than in the first experiment, so that a disturbance of metabolism such as might be caused by the alcohol would affect a smaller quantity of the precursors of uric acid in the latter case.

Again, as in Experiment 1, there is a marked increase in the excretion of ammonia. The diet was uniform throughout, and the writer is unable to account for such an increase, except by supposing that the alcohol has caused changes in metabolism resulting in the production of an increased amount of acid which is excreted as an ammonium salt.

Experiment 2 b. — The after period of the second experiment closed Dec. 8. On Dec. 9 and 10 the same diet was continued, and whiskey in the form of "High Balls" was added. These "High Balls" contained 40 c.c. of whiskey each, and were taken at 4 P. M., 5 P. M., 8 P. M., and 9 P. M. The after period of the whiskey experiment thus served as the fore period for the "High Ball" experiment.

The results show again an increase in the ammonia and uric acid excretion, and this is very near that obtained in the whiskey period of Experiment 2 a.

Experiment 3. — In this experiment, which was carried out upon the same subject (A) as those already given, the object was to determine the effect of taking pure alcohol upon the hourly excretion of the endogenous uric acid. The supper on the day before the experiment began was light and contained no purin food. No breakfast was eaten on the day of the experiment, nor was any food taken during the experimental period. 50 c.c. of water taken each hour. Urine collected each hour, beginning at 8 A. M. At 10 A. M., 50 c.c. of absolute alcohol, diluted with water to 200 c.c., were taken. This alcohol was quickly absorbed, and was sufficient

in amount to produce marked narcosis, which was most profound shortly after 11 A. M. No urine was lost, however, the subject being aroused by an assistant. Quick recovery was made, being complete at 1 P. M., and no bad or unpleasant after effects were noticed.

TABLE III.
SUMMARY OF EXPERIMENT 3.

Hour.	Volume.	Uric acid.
	c.c.	milligrams
7-8	57	30.4
8-9	84	33.0
9-10	110	31.5
10-11 *	465	26.5
11-12	565	29.2
12-1	57	27.8
1-2	35	28.8
2-3	30	24.0
3-4	20	20.8
* Alcohol taken at 10 A. M.		

There is here no increase in uric acid excretion. The output had fallen to a nearly constant level at 8 A. M. Since the excreted acid was then of endogenous origin, and since so large a quantity of alcohol as 50 c.c. taken at one dose failed to cause an increase, it seems probable that deranged metabolism of purin food is an important cause of the increase noted in the previous experiments.

Another point of interest is the fact that the strong diuresis caused by the alcohol has had no effect upon the uric acid excretion. Sweeping urates out of the system by increasing the volume of water excreted finds little support in this experiment.

Experiment 4.—Since the endogenous uric acid was not increased by the alcohol, further experiments were made to determine the effect of taking alcohol with food. The first in this series, Experiment 4, lasted three days. The first day served for control. On the night before the experiment began, a light meal containing no purin food was eaten, and no breakfast was eaten the next morning. At 1 P. M. the experimental meal was eaten, and the quantity and quality of the food taken at this meal was the same for the three days.

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On the first alcohol day, 35 c.c. absolute alcohol diluted with water were taken with the test meal. On the second alcohol day, 20 c.c. alcohol were taken one hour after the meal, and 5 c.c. each hour thereafter until the close of the experimental period.

TABLE IV.
SUMMARY OF EXPERIMENT 4.

CONTROL DAY.			FIRST ALCOHOL DAY.			SECOND ALCOHOL DAY.		
Hour.	Vol.	Uric acid.	Hour.	Vol.	Uric acid.	Hour.	Vol.	Uric acid.
	c.c.	milligrams		c.c.	milligrams		c.c.	milligrams
11	46.0	22.5	11	30	15.0	11	20	8.9
12	26.0	17.8	12	16	10.7	12	17	11.2
1*	20.0	18.7	1*	15	7.0	1	16	9.8
2	16.0	10.3	2	17	9.8	2*	18	10.7
3	17.0	15.0	3	18	16.8	3	23	13.1
4	19.0	19.2	4	20	22.5	4	27	21.0
5	25.0	24.3	5	28	27.6	5	35	35.1
6	20.0	22.9	6	34	35.1	6	24	32.5
7	20.0	21.0	7	25	29.0	7	41	29.5
8	8	22	20.1	8	71	29.0

* Meal hour.

EXPERIMENTAL MEAL.

Ham, 100 grams; bread, 75 grams; butter, 25 grams; coffee, 400 c.c.; banana, 100 grams.

The results show that the alcohol has had a different effect here (Table IV.) than was obtained in Experiment 3 on the endogenous uric acid. There was a sharp increase in the excretion of urates as compared with the control day, and this increase reached its maximum 4-5 hours after the meal. On the second day, the maximum point was reached earlier, and the curve does not drop as sharply as on the first alcohol day, a difference which is to be explained by the repeated small doses (5 c.c.) of alcohol.

Experiment 5. — In the preceding experiment the alcohol caused an increase in the excretion of uric acid, but the objection may be raised that this was a mere hastening of the normal output, thereby leaving a smaller

quantity to be excreted later. Therefore in the present experiment the total urine for twenty-four hours was collected in order to determine this point.

The meal taken on the night before the experiment was of non-purin food. No breakfast was taken on the experimental days. At 12 M. the same kind of a test meal as in Experiment 4 was eaten. Urine collected in hourly periods beginning at 8 A. M. and continuing until 6 P. M. From 6 P. M. until 7 A. M. the following morning, the urine was collected in total, thus completing the experimental day.

At 12 M. on the alcohol day, 30 c.c. of absolute alcohol suitably diluted with water were taken with the test meal.

TABLE V.
SUMMARY OF EXPERIMENT 5.

CONTROL DAY.			ALCOHOL DAY.		
Hour.	Volume.	Uric acid.	Hour.	Volume.	Uric acid.
	c.c.	gram		c.c.	gram
8	57	0.019	8	38	0.017
9	63	0.017	9	45	0.016
10	165	0.018	10	38	0.013
11	23	0.013	11	92	0.017
12 *	36	0.020	12	47	0.016
1	30	0.017	1	30	0.014
2	34	0.016	2	100	0.013
3	25	0.015	3	45	0.019
4	26	0.018	4	50	0.021
5	31	0.026	5	90	0.029
6	36	0.017	6	60	0.024
			Control day.		Alcohol day.
Residue of 24 hours (6 P. M.-7 A. M.)			gram 0.206		gram 0.247
Total uric acid			0.402		0.446
* Meal hour.					

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There was an increase of about 10 per cent for the twenty-four hours which was not a mere hastening of the normal output during the few hours immediately following the meal.

TABLE VI.
SUMMARY OF EXPERIMENT 6.

Date.	Volume.	Nitrogen.	Uric acid.	Wine.
	c.c.	GRAMS	GRAM	c.c.
Dec. 19	1500	13.94	0.500	
" 20	1400	14.26	0.524	
" 21	1120	15.51	0.556	
" 22	1360	18.48	0.655	
" 23	1500	18.16	0.567	
" 24	1480	16.58	0.824	500
" 25	1400	17.11	0.722	500
" 26	1340	15.42	0.570	
" 27	1260	16.15	0.612	
" 28	1220	16.58	0.617	
" 29	1310	18.06	0.573	
" 30	1340	16.89	0.529	
" 31	1400	14.26	0.672	500
" 1	1340	14.47	0.612	500
" 2	1340	16.94	0.573	
" 3	1540	16.94	0.612	
AVERAGE OF DAILY RESULTS.				
		Nitrogen.	Uric acid.	
		GRAMS	GRAM	
Fore period		16.07	0.560	
First alcohol period		16.84	0.773	
After period		16.64	0.580	
Second wine period		14.36	0.642	
Second after period		16.94	0.594	

Experiment 6.—Port wine has the reputation of being more troublesome to gouty persons than other alcoholic fluids. It is conceivable that this bad effect may not be due to the alcohol contained in the wine so much as other components, such as extractives and organic acids. Therefore a metabolism experiment was carried out on Subject A, in which the alcohol was taken in form of port wine containing 15.5 per cent alcohol. The same general plan and the same diet were used as in Experiment 2.

A striking increase in uric acid excretion was found; in the first wine period 38 per cent, and in the second 10.6 per cent (Table VI). During the three days just preceding the first wine period the subject had a slight cold which reached a climax on the first alcohol day. Possibly this may be a factor in explaining the unusual increase of 38 per cent in the uric acid excretion.

Experiment 7.—The experiment using port wine as the alcoholic fluid was repeated upon the same subject (A) some weeks later. In this instance excellent health was enjoyed throughout, and the variations noted cannot be attributed to anything but the wine.

TABLE VII.
SUMMARY OF EXPERIMENT 7.

Date.	Volume.	Nitrogen.	Uric acid.	Wine.
	c.c.	grams	gram	c.c.
Feb. 21	1000	10.56	0.516	
" 22	1120	12.88	0.498	
" 23	920	13.21	0.528	
" 24	890	12.88	0.696	350
" 25	890	12.90	0.810	350
" 26	1000	14.25	0.552	
" 27	1240	15.48	0.576	
ANALYSIS OF COMPOSITE SAMPLES.				
		Xanthin bases.	Uric acid.	
		gram	gram	
Fore period		0.0433	0.514	
Alcohol period		0.0630	0.764	
After period		0.0439	0.564	

Effect of Alcohol upon the Excretion of Uric Acid. 25

An extraordinary increase of 46.5 per cent in the uric acid excretion was obtained (Table VII). Nearly the same increase, 42.5 per cent, was found in the purin bases reckoned as xanthin. Probably both are due to the same cause, and the conditions indicate this to be a toxic effect of the wine interfering with the oxidative processes presumably of the liver. This result is in striking contrast to the results obtained by Mandel,¹ who found that in the aseptic fevers an increase of purin bases was accompanied by a decrease in the uric acid excretion. It is probable that both the purin bases and uric acid were of endogenous origin in the cases he studied, the diet immediately following the operation being nearly purin free.

The clinical judgment in regard to port wine was verified, for 350 c.c. of wine containing 52.5 c.c. of alcohol caused a much greater effect than a larger quantity of alcohol taken in a purer form.

All the experiments thus far quoted were made upon the same subject (A), the writer. In order to determine whether the behavior toward alcoholic fluids which was found in his case was an idiosyncrasy, experiments were performed upon other subjects. The same plan was followed as in the experiments already given. On the night before the experiment began a light meal containing no purin food was eaten; no breakfast was eaten on the day of the experiment; the test meal of the control day was like that of the alcohol day; on the evening of the experimental days a light meal containing no purin food was taken. Various alcoholic fluids were used, and the quantity was in no case excessive.

Since these experiments are alike, they may be discussed together. The results are given on the following pages.

¹ MANDEL: This journal, 1904, x, p. 990.

Experiment 8.— Hourly period experiment in the excretion of uric acid.
 Subject B. On the alcohol day, 700 cubic centimetres of India Pale Ale containing 8 per cent alcohol were taken with the test meal.

TABLE VIII.
 SUMMARY OF EXPERIMENT 8.

CONTROL DAY.			ALCOHOL DAY.	
Hour.	Volume.	Uric acid.	Volume.	Uric acid.
	c.c.	milligrams	c.c.	milligrams
9	58	18	40	26
10	46	19	26	16
11	40	21	30	17
12	40	21	25	15
1 *	26	12	23	10
2	18	10	33	17
3	23	18	30	22
4	30	17		
5	32	15	24	21
6	28	17	28	36
7	34	32	32	22
RESIDUE OF 24 HOURS.				
Control day.			Alcohol day.	
Volume 380 c.c.			Volume 455 c.c.	
Uric acid 226 mgms.			Uric acid 264 mgms.	
* Meal hour.				

Experiment 9.—Hourly period experiment in the excretion of uric acid. The usual plan was followed. 150 c.c. of port wine containing 15 per cent by volume of alcohol were taken with the test meal on the alcohol day. Subject D.

TABLE IX.
SUMMARY OF EXPERIMENT 9.

CONTROL DAY.			ALCOHOL DAY.	
Hour.	Volume.	Uric acid.	Volume.	Uric acid.
	c.c.	milligrams	c.c.	milligrams
9	60	12	34	17
10	300	4	40	17
11	60	13	60	20
12 *	30	10	50	16
1	22	9	27	14
2	54	15	73	20
3	36	22	35	35
4	37	22	26	26
5	48	24	51	31
6	64	29	51	30

* Meal hour.

Experiment 10.—Hourly excretion of uric acid as affected by alcohol. 350 c.c. of ale containing 8.44 per cent by volume of alcohol are taken with the test meal on the alcohol day, Subject A.

TABLE X.
SUMMARY OF EXPERIMENT 10.

CONTROL DAY.			ALCOHOL DAY.	
Hour.	Volume.	Uric acid.	Volume.	Uric acid.
	c.c.	milligrams	c.c.	milligrams
8	60	28.5	64	21
9	65	25	60	19
10	35	17	90	25
11	33	18	85	25
12	26	17	93	18
1 *	18	12	42	15
2	17	10	88	25
3	27	13	165	26
4	36	26	40	25
5	37	29	40	25
6	43	25	48	35
7	43	25	44	24
* Meal hour.				

Experiment 11. — Hourly excretion of uric acid as affected by alcohol. 150 c.c. of port wine containing 15 per cent by volume of alcohol were taken with the test meal on the alcohol day, Subject E.

TABLE XI.
SUMMARY OF EXPERIMENT II.

CONTROL DAY.			ALCOHOL DAY.	
Hour.	Volume.	Uric acid.	Volume.	Uric acid.
	c.c.	milligrams	c.c.	milligrams
8	26	18	35	30
9	27	18	26	26 *
10	27	21	26	40
11	25	21	18	26
12 *	24	23	18	26
1	24	21	22	21
2	32	34	38	36
3	31	34	44	45
4	31	34	45	44
5	40	29	50	44

RESIDUE FOR THE 24 HOURS.	
Control day.	Alcohol day.
Volume 300 c.c.	Volume 350 c.c.
Uric acid 261 mgms.	Uric acid 248 mgms.
Total uric acid 538 mgms.	Total uric acid 618 mgms.

* Meal hour.

AVERAGE RESULTS FROM EXPERIMENTS 4, 5, 8, 9, 10, 11.

The following figures given in Table XII were obtained by averaging the results of the six experiments on the influence of alcoholic fluids upon the hourly excretion of uric acid.

TABLE XII.

CONTROL DAY.		ALCOHOL DAY.
Hour.	Uric acid.	Uric acid.
	milligrams	milligrams
9	18.9	19.0
10	15.7	16.3
11	17.1	16.4
12 *	16.1	14.4
1	12.8	16.2
2	15.6	19.5
3	19.3	25.7
4	23.6	28.3
5	24.8	34.2
6	25.5	28.8
RESIDUE OF 24 HOURS.		
Control day.		Alcohol day.
Uric acid . . 338 mgms.		Uric acid . . 442 mgms.
* Meal hour.		

The results show that an increase of uric acid excretion is in most cases one of the effects of taking alcohol. This increase reaches its maximum five hours after the meal and alcohol have been taken. Other investigators have found that the maximum increase as a result of taking food occurs at the same time, so that an additional argument is afforded for regarding the increase as caused by the alcohol as due to a disturbed metabolism of the uric acid precursors found in the food.

Personal idiosyncrasy is, however, of some importance in this behavior toward alcohol, as it is in metabolic processes in general. The experiment given on the following page is the only one in the whole series that was carried out for this paper in which an increase of uric acid excretion did not follow the taking of alcohol. In both the experiments on Subject C, a smaller quantity of uric acid was excreted immediately following the meal on the alcohol day than on the control day; and the total quantity for twenty-four hours was smaller on the alcohol day.

It will be observed, however, that even in this case the amount of uric acid excreted between 6 P.M. and 8 P.M. the following morning was considerably greater on the alcohol day. The same plan was observed in this experiment as in the others, and the only explanation for such a divergence from the results obtained under similar conditions with other subjects is the personal idiosyncrasy of Subject C.

Experiment 12. — Hourly period experiment on the excretion of uric acid as affected by alcohol. On the alcohol day, 700 c.c. of India Pale Ale containing 8 per cent by volume of alcohol were taken with the test meal, Subject C (Table XIII).

TABLE XIII.
SUMMARY OF EXPERIMENT 12.

CONTROL DAY.			ALCOHOL DAY.	
Hour.	Volume.	Uric acid.	Volume.	Uric acid.
	c.c.	milligrams	c.c.	milligrams
9	24	13	31	20
10	21	14	32	21
11	23	13	28	21
12*	17	9	19	11
1	20	14	45	15
2	38	40	425	25
3	39	32	265	26
4	39	46	50	26
5	50	50	50	27
6	47	51	50	24
RESIDUE FOR THE 24 HOURS.				
Control day.			Alcohol day.	
Volume 550 c.c.			Volume 540 c.c.	
Uric acid 312 mgms.			Uric acid 358 mgms.	
Total uric acid 596 mgms.			Total uric acid 575 mgms.	
* Meal hour.				

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Experiment 13.— Subject C. On the alcohol day 300 c.c. of port wine containing 15 per cent by volume of alcohol were taken with the test meal (Table XIV).

TABLE XIV.
SUMMARY OF EXPERIMENT 13.

CONTROL DAY.			ALCOHOL DAY.	
Hour.	Volume.	Uric acid.	Volume.	Uric acid.
	c.c.	milligrams	c.c.	milligrams
10	62	27	46	28
11	32	16	28	15
12*	25	13	22	13
1	26	14	20	13
2	20	10	110	11
3	23	17	73	22
4	36	33	38	8
5	36	26	72	20
6	28	23	56	16
* Meal hour.				

Two metabolism experiments were carried out on different subjects, A and F, in which alcohol was taken in the form of ale. The same plan was followed as in the previous metabolism experiments, great care being taken to have a uniform diet throughout.

The results are shown in Table XV, for Experiment 14, and Table XVI, for Experiment 15. They show in each case a marked increase of uric acid excretion during the alcohol period. The experiments differ in no essential way from those previously carried out, in which alcohol in other forms was used, and they simply afford additional evidence of its action.

Experiment 14.— Metabolism experiment, Subject A. Alcohol in the form of an ale containing 8.44 per cent by volume of alcohol was used. The same general plan was followed as in the previous metabolism experiments.

TABLE XV.
SUMMARY OF EXPERIMENT 14.

Date.	Volume.	Nitrogen.	Uric acid.	Ale.
	c.c.	grams	gram	c.c.
Feb. 23	1060	11.40	0.354	
" 24	1240	13.41	0.432	
" 25	1370	14.64	0.516	
" 26	1000	14.65	0.528	
" 27	1200	14.78	0.516	
" 28	1700	14.11	0.625	1050
" 1	1400	13.94	0.768	1050
" 2	1460	13.19	0.612	1050
" 3	1260	14.04	0.738	1050
" 4	1320	14.15	0.558	
" 5	1120	14.04	0.564	
" 6	1000	14.16	0.540	
DAILY AVERAGES.				
		Nitrogen.	Uric acid.	
		grams	gram	
Fore period		14.65	0.520	
Alcohol period		13.82	0.685	
After period		14.12	0.554	
RESULTS OBTAINED BY ANALYSIS OF COMPOSITE SAMPLES.				
	Fore period.	Alcohol period.	After period.	
	grams	grams	grams	
Total nitrogen	14.460	13.190	13.620	
Urea nitrogen	12.770	11.870	12.260	
Uric acid	0.522	0.645	0.516	
Total phosphates	1.950	2.175	2.220	
Total sulphates	2.700	3.118	2.752	
Combined sulphates	0.214	0.214	0.201	

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Experiment 15.—Alcohol in the form of ale containing 8.44 per cent by volume of alcohol was used in this experiment, Subject F.

TABLE XVI.
SUMMARY OF EXPERIMENT 15.

Volume.	Nitrogen.	Uric acid.	Ale.
c.c.	grams	gram	c.c.
1080	10.56	0.378	
820	11.19	0.390	
1040	12.75	0.432	
1500	13.08	0.516	1050
1100	12.77	0.490	1050
860	12.45	0.402	
COMPOSITE SAMPLES.			
		Fore period.	Alcohol period.
		grams	grams
Nitrogen		11.410	12.740
Urea		10.700	11.700
Uric acid		0.393	0.498
Total phosphates		1.905	2.400
Total sulphates		2.239	2.809

CONCLUSIONS.

Some of the metabolism experiments give evidence of the proteid-sparing effect of alcohol; for instance, in Experiment 13 the average daily nitrogen of the fore period was 14.65 grams; of the alcohol period, 13.82 grams, of the after period, 14.12 grams. It will be recalled, however, that the proteid-sparing effect appears with a person unaccustomed to alcohol only after the alcohol has been taken for some days; and the conditions of the writer's experiments prevented the appearance of this effect. But Experiment 13 was carried out upon a subject (A) who, though unaccustomed to alcohol in the beginning, had served in a number of experiments, and had thus reached a condition of partial immunity to the toxic effects of the alcohol.

After a consideration of these experiments, it hardly seems possible to doubt that alcohol, even in what is considered by the most conservative as a moderate amount, causes an increase in the excretion of uric acid. And this effect is seen almost immediately after taking the alcohol.

The following points indicate that the effect is due to a toxic effect on the liver, thereby interfering with the oxidation of the uric acid derived from its precursors in the food:

1. Alcohol taken without food causes no increase. See Experiment 3.
2. In Experiment 2, the diet contained much less purin food than in Experiment 1, and there was a smaller increase in excretion.
3. The maximum increase occurs at the same time after a meal as it does when purin food but no alcohol is taken.
4. The purin bases are affected to the same degree as the uric acid.
5. Alcohol is rapidly absorbed and passes at once to the liver, the organ which has most to do with the metabolism of proteid cleavage products.

There is no evidence that the alcohol has merely hastened the excretion of urates normally present in the blood; the increased excretion means that a larger quantity has been in circulation, and although it is classed by Von Noorden¹ among the substances easily excreted, still most physiologists would consider the presence in the blood of this larger quantity as undesirable. Certainly in pathological conditions it might be harmful.

If we accept the origin of the increased quantity of uric acid to be in the impaired oxidative powers of the liver, the results of these experiments will have greater significance than can be attributed to uric acid alone. For the impaired function would affect other processes which are normally accomplished by that organ, and the possibilities for entrance into the general circulation of toxic substances, of intestinal putrefaction, for instance, would be increased. The liver performs a large number of oxidations and syntheses designed to keep toxic substances from reaching the body tissues, and if alcohol, in the moderate quantity which caused the increase in uric acid excretion, impairs its power in this respect, the prevalent ideas regarding the harmlessness of moderate drinking need revision.

¹ VON NOORDEN: *Diseases of Metabolism and Nutrition*, nephritis, 1904, p. 44.

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Alcohol is a food in the sense that when used in small quantities the energy from its oxidation may be used for some of the body needs; but since, at the same time, it interferes with the normal activities of a most important organ, its food-value may be overbalanced by its toxic effect. Salt water may be used in a steam-boiler, and the steam from its evaporation may transmit the energy of the fuel to the revolving wheels, but its corrosive action on the steel forbids its use, like alcohol, except in emergencies.

The writer is much indebted to Professor Chittenden and Professor Mendel for many helpful suggestions during the course of the work.

THE ELIMINATION OF ENDOGENOUS URIC ACID.

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THE intimate relationship between the so-called purin constituents of the diet and the output of uric acid is too firmly established at the present time to require detailed discussion.¹ It is likewise well known that the elimination of uric acid does not cease during hunger or after the ingestion of foods which are free from purin components. It is obvious that in these cases the uric acid must have its origin in tissue constituents; the purin derivatives of disintegrating cells rich in nucleic acid compounds at once suggest themselves as precursors of the eliminated substance. Burian and Schur² have attempted to distinguish between *endogenous* and *exogenous* urinary purin constituents, the latter term being applied to that fraction of the total output which has its origin in the purin bodies of the foods, while the endogenous component is referred to tissue metabolism. As the result of their extensive investigations, Burian and Schur come to the conclusion that the endogenous component of the eliminated urinary purin compounds is constant for each individual, that it is, in other words, an individual factor.

Mareš³ had earlier concluded that the elimination of uric acid reaches a constant for every individual, although the individual differences may vary widely. His publications date from a period when the genetic relation between the purin compounds and uric acid was little understood. Schreiber and Waldvogel⁴ had also assumed a

¹ The enormous literature on the subject has been reviewed in monographs by SCHREIBER: *Die Harnsäure*, Stuttgart, 1899; WALKER HALL: *The Purin Bodies of Food Stuffs*, 1903; WIENER: *Ergebnisse der Physiologie*, 1902, i, Part 1, p. 355; *Ibid.*, 1903, ii, Part 1, p. 377; BURIAN and SCHUR: *Archiv für die gesammte Physiologie*, 1900, lxxx, p. 241.

² BURIAN and SCHUR: *Loc. cit.*, also *Archiv für die gesammte Physiologie*, lxxvii, p. 239.

³ MAREŠ: *Archives slaves de biologie*, 1888, iii, p. 207.

⁴ SCHREIBER and WALDVOGEL: *Archiv für experimentelle Pathologie und Pharmacie*, 1899, xlii, p. 69.

constant endogenous output of uric acid, as the result of observations on starving men. The criticism has, however, properly been offered that the data obtained during inanition — when a continued breaking down of cellular tissue may reasonably be assumed to take place — are scarcely to be accepted as “normal” or “physiological” values. The conclusions of Burian and Schur with reference to the constancy of the endogenous purin output are based on a large number of observations upon men living upon a strictly purin-free diet. SIVÉN¹ likewise found a remarkable constancy in his output of uric acid during periods in which the purin-free diet was widely varied in quantity and composition. Loewi² has assumed that the endogenous output of uric acid is dependent in a measure on the food ingested, — a conclusion which has, in turn, been subjected to rigorous criticism.³ Finally Kaufmann and Mohr⁴ favor the theory of the individual constancy of uric acid, publishing experimental data in confirmation. They believe, in distinction from Burian and Schur, that the endogenous nuclein metabolism can be diminished by increased ingestion of non-nitrogenous food, — “durch Calorienüberfütterung,” as they express it.

“The sources of endogenous purins are probably numerous, and the quantities derived from each may vary with the hourly activities and daily needs. Although our present knowledge upon the point is somewhat inadequate, we may be sure that in pathological conditions alterations in any one of the factors may lead to diminution or increase of endogenous purins. So far as experimental results can suggest normal action, one portion of the total endogenous purins is broken down to urea, and the remainder excreted as uric acid. Abnormal endogenous purin metabolism may, therefore, consist in an increased production with excessive or diminished destruction. Hence arises the difficulty of any correct inference from the results of endogenous purin elimination. Constancy of endogenous purin excretion points to normal metabolism and the maintenance of the several factors concerned. Variations in the endogenous urinary purin of the same individual upon a fixed diet indicates altered relations of the contributory

¹ SIVÉN: Skandinavisches Archiv für Physiologie, 1901, xi, p. 132.

² LOEWI: Archiv für experimentelle Pathologie und Pharmacie, 1900, xlv, p. 1; Archiv für die gesammte Physiologie, 1902, lxxxviii, p. 296.

³ Cf. BURIAN and SCHUR; also WIENER: *Loc. cit.*

⁴ KAUFMANN and MOHR: Deutsches Archiv für klinische Medizin, 1902, lxxiv, pp. 141, 348.

functions."¹ The preceding quotation from Walker Hall, influenced largely by the work of Burian, indicates the importance of determining definitely the question regarding the constancy of endogenous uric acid output and its dependence upon the individual and his daily disposition. The following experiments were undertaken as a contribution in this direction. The selection of a purin-free diet was obviously of primary importance. It is well known that foods of animal origin, as a class, contain much more of the purin compounds than do the vegetable foods. Among the latter we find, from the analyses of Burian and Schur (*loc. cit.*), and of Hall (*loc. cit.*), that rice, eggs, milk, butter, cheese, and preparations of fine wheat, such as bread, contain none or but very minute quantities. Most of the older experiments on uric acid formation have a lessened value because of a non-recognition of these facts. In many instances where some substance was studied as to its effect upon the production or elimination of uric acid, too little attention has been paid to providing a basal diet which in itself would not serve as a source of the acid. The plan followed in the present trials was to have the subject select from the purin-free foods such a diet as he could endure and, having proved this by trial, to take the same kind and amount of food and at the same times of day as long as the test lasted. Trials were made at different times with the same subject, using the same and also different foods; two subjects were tested with a common diet, and the influence of other factors, such as labor, increased food-materials, or water were studied. The analyses of the urine were conducted as follows: nitrogen was determined by the Kjeldahl process; uric acid by Folin's method; phosphoric acid (P_2O_5) by titration with uranium acetate.

EXPERIMENTS.

Subject A, First Series.—The subject was a healthy male of 42 years, weighing about 55 kilos. During the first nine days the daily diet consisted of:

Milk	1350 c.c.
" Force "	35 gms.
Cream	50 "
Sugar	20 "
Oyster crackers	250 "
Cheese	30 "

¹ WALKER HALL: *Loc. cit.*, p. 114.

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Eggs	96 gms.
Apple	90 "
Wheat bread	25 "
Butter	15 "
Estimated fuel value	2770 calories.

On the following days there were added to the preceding ration :

Wheat bread	25 gms.
Butter	15 "
Barley-sugar candy	50 "
Total fuel value	3160 calories.

COMPOSITION OF THE URINE.

Day.	Nitrogen.	Uric acid.	P ₂ O ₅ .
	grams	gram	grams
1	11.32	0.351	1.65
2	12.04	0.387	1.95
3	12.86	0.342	2.24
4	11.96	0.335	2.39
5	11.18	0.313	2.69
6	12.86	0.310	2.64
7	12.04	0.258	2.53
8	11.69	0.305	2.38
9	12.58	0.212	2.52
10	12.32	0.304	2.72
11	11.69	0.305	2.38
12	11.85	0.300	2.62
13	11.56	0.294	2.51
Daily av.	11.99	0.308	2.41

The average daily uric acid output for the first period of nine days was **0.311 gm.**, and of the second period (the last four days) was **0.301 gm.**

Subject A, Second Series, and Subject B, First Series. — This experiment was undertaken to learn the effect of the same diet on the uric acid output of two subjects, the experiments being carried on simultaneously. Subject B was a female, aged 20, weighing, with clothing, 59 kilos. Subject A weighed, with clothing, 56 kilos. During the first seven days the diet consisted of :

Milk	1350 c.c.
"Force"	35 gms.
Sugar	20 "
Oyster crackers	250 "
Cheese	30 "
Eggs	96 "
Apples	90 "
Wheat bread	50 "
Butter	15 "
Estimated fuel value	2736 calories.

On the eighth day and thereafter there were added :

Confectionery	100 gms.
Total fuel value	3146 calories.

COMPOSITION OF THE URINE.

Day.	SUBJECT A.			SUBJECT B.		
	Nitrogen.	Uric acid.	P ₂ O ₅ .	Nitrogen.	Uric acid.	P ₂ O ₅ .
	grams	gram	grams	grams	gram	grams
1	12.59	2.35
2	11.47	0.313	2.78	12.12	0.374	2.57
3	11.89	0.315	2.79	12.74	0.355	2.54
4	10.72	0.315	2.63	12.09	0.331	2.36
5	11.82	0.267	2.93	10.68	0.336	2.12
6	12.21	0.298	2.65	10.04	0.314	2.00
7	12.19	0.323	2.54	10.89	0.376	2.48
8	12.20	0.295	2.53	12.10	0.327	2.52
9	10.75	0.322	2.64
10	11.90	0.307	2.67	10.88	0.321	2.37
11	10.90	0.300	2.35	10.99	0.327	2.38
12	11.16	0.298	2.58
Daily av.	11.58	0.305	2.86	11.39	0.340	2.37

The average excretion of uric acid from the first to the seventh day was, for Subject A, **0.305 gm.**; for Subject B, **0.348 gm.** For the last period, with increased diet, it was, for Subject A, **0.305 gm.**; for Subject B, **0.325 gm.**

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Subject C, First Series. — The subject was a male, aged 28, and weighing at the beginning, including clothing, 76.7 kilos; on the fourth day, the same; on the ninth day, 75.7 kilos, after severe exercise; on the thirteenth day, 76.2 kilos. The daily diet consisted of:

Wheat bread	341 gms.
Eggs	114 "
Cheese (whole milk)	57 "
" Force "	85 "
Butter	42 "
Sugar	71 "
Apples	284 "
Milk	225 c.c.
Calculated fuel value	2577 calories.

On each of the last four days there was eaten, in addition to the above:

Maple sugar	150 gms.
Total fuel value	3085 calories.

COMPOSITION OF THE URINE.

Day.	Nitrogen.	Uric acid.	P ₂ O ₅ .	
	GRAMS	GRAM	GRAMS	
1	13.56	0.428	2.20	
2	13.86	0.421	2.27	
3	0.510	2.54	
4	13.02	0.458	2.12	
5	0.466	2.23	Gymnasium exercise; severe.
6	13.93	0.512	2.25	
7	14.47	0.520	2.30	
8	12.76	0.475	1.94	Gymnasium exercise.
9	10.84	0.381	1.88	Skating; severe exercise.
10	16.86	0.589	2.62	" "
11	11.66	0.464	2.09	
12	14.05	0.544	2.43	
13	12.53	0.448	1.86	
Daily av.	13.41	0.478	2.21	

The average uric acid output for the four days of severe exercise was **0.478 gm.**, and for the last four days, when on an increased diet, was **0.485 gm.**

Subject A, Third Series, and Subject C, Second Series.—The daily amount of uric acid excreted by these subjects on a purin-free diet having been proven to be widely different, another dietary was selected which was a compromise between the two original ones. It contained :

Milk	900 c.c.
Wheat bread	170 gms.
Butter	43 "
Sugar	57 "
" Malta Vita "	57 "
Cheese (whole milk)	28 "
Eggs	100 "
Oyster crackers	113 "
Apples	250 "
Estimated fuel value	2710 calories.

During the evenings of the fourth, fifth, and sixth days, Subject C engaged in very severe exercise in the gymnasium (wrestling against two opponents) until he was thoroughly tired out. The products of this

COMPOSITION OF THE URINE.

Day.	SUBJECT A.			SUBJECT C.		
	Nitrogen.	Uric acid.	P ₂ O ₅ .	Nitrogen.	Uric acid.	P ₂ O ₅ .
	grams	gram	grams	grams	gram	grams
1	10.78	0.340	1.88	12.97	0.414	1.97
2	10.60	0.300	2.20	15.20	0.403	2.25
3	10.87	0.320	2.19	14.25	0.453	2.75
4	11.55	0.341	2.25	13.77	0.452	2.59
5	12.07	0.346	2.19	13.66	0.573	2.46
6	11.38	0.343	2.29	13.18	0.525	2.32
7	10.92	0.261	2.22	13.06	0.465	2.69
8	10.36	0.271	2.07	13.83	0.431	2.53
9	11.82	0.317	2.40	14.91	0.409	2.59
10	11.16	0.308	2.36	14.42	0.401	2.75
Daily av.	11 15	0.315	2.21	13.92	0.452	2.49

metabolism ought to appear in the urine of the next day. His weight, with clothing, was at the beginning, 76.3 kilos; on the fourth day, 76 kilos; after wrestling, on the seventh day, 74.5 kilos; and on the tenth day, 75.1 kilos. Subject A weighed, with clothing, 56.7 kilos at the beginning, and 56.1 kilos at the end of the trial.

The average daily uric acid output during severe exercise was, for Subject C, **0.513** gm.

Subject A, Fourth Series. — In this the diet was the same as in the first part of the first series. In each of the last three days of this series there was taken, in addition, 700 c.c. of distilled water.

COMPOSITION OF THE URINE.

Day.	Nitrogen.	Uric acid.	P ₂ O ₅ .
1	grams 11.89	gram 0.363	grams 1.95
2	10.09	0.263	1.81
3	13.19	0.344	2.47
Average for first three days	11.73	0.324	2.08
4	13.99	0.304	2.48
5	13.77	0.342	2.50
6	12.81	0.309	2.47
Average for last three days	13.52	0.318	2.48
Average for whole series	12.63	0.321	2.28

Subject A, Fifth Series. — The food eaten was the same as in the fourth series on the same subject. In order to make the night and day periods correspond as nearly as possible with the time of sleep and waking respectively, they were made to extend from 7.30 A. M. to 10.30 P. M. for the day, and from 10.30 P. M. to 7.30 A. M. for the night period. The day, in consequence, contained fifteen hours and the night period, nine hours. The nitrogen, phosphoric acid, and uric acid excreted during these periods were determined and the total divided by fifteen or nine, to get the hourly excretion.

COMPOSITION OF THE URINE.

DAY.	DAY URINE, PER HOUR.			NIGHT URINE, PER HOUR.			TOTAL URINE.		
	Nitro- gen.	Uric acid.	P ₂ O ₅ .	Nitro- gen.	Uric acid.	P ₂ O ₅ .	Nitro- gen.	Uric acid.	P ₂ O ₅ .
1	gram 0.485	gram 0.0138	gram 0.092	gram 0.512	gram 0.0106	gram 0.090	grams 11.87	gram 0.303	grams 2.20
2	0.572	0.0161	0.108	0.458	0.0089	0.085	12.70	0.321	2.39
3	0.521	0.0135	0.109	0.532	0.0095	0.106	12.60	0.289	2.59
4	0.535	0.0148	0.110	0.499	0.0104	0.089	12.48	0.311	2.42
5	0.543	0.0143	0.115	0.564	0.0124	0.090	13.23	0.325	2.63
6	0.540	0.0145	0.111	0.530	0.0117	0.102	12.87	0.323	2.57
7	0.546	0.0156	0.109	0.530	0.0113	0.098	12.96	0.336	2.52
8	0.537	0.0142	0.111	0.519	0.0090	0.101	12.72	0.294	2.57
Daily av.	0.535	0.0146	0.108	0.518	0.0105	0.095	12.68	0.313	2.49

Subject A, Sixth Series. — During a course of dieting for another purpose, the food being purin-free, determinations were made of the daily nitrogen, uric acid, and phosphoric acid output. Although only two determinations were made, their agreement with the others permits their introduction. They represent the amounts excreted in the fourth and fifth days of the test, of which the remainder were not determined. The diet consisted of:

Milk 1350 c.c.
 Oatmeal 75 gms.
 Wheat crackers 180 "
 Sugar 100 "
 Shredded wheat biscuit 30 "
 Estimated fuel value 2536 calories.

COMPOSITION OF THE URINE.

Day.	Nitrogen.	Uric acid.	P ₂ O ₅ .
1	grams 10.30	gram 0.296	grams 2.25
2	9.95	0.300	1.97
Daily av.	10.08	0.298	2.11

Subject D. — This was a university student, male, weighing at the commencement of the series 65.7 kilos, with clothing, and at the end, 65.9 kilos. During the time of dieting the weight did not vary more than half a kilo from these figures. The diet for the first twelve days consisted of:

Milk	1125 c.c.
" Force "	50 gms.
Wheat crackers	50 "
Wheat bread	250 "
Sugar	30 "
Butter	35 "
Cheese	30 "
Estimated fuel value	2400 calories.

COMPOSITION OF THE URINE.

Day.	DAY URINE, PER HOUR.			NIGHT URINE, PER HOUR.			TOTAL URINE.			
	Nitro- gen.	Uric acid.	P ₂ O ₅ .	Nitro- gen.	Uric acid.	P ₂ O ₅ .	Nitro- gen.	Uric acid.	P ₂ O ₅ .	
	gram	gram	gram	gram	gram	gram	grams	gram	grams	
1	0.522	0.0153	0.112	0.478	0.0153	0.126	12.18	0.374	2.79	
2	0.636	0.0199	0.101	0.557	0.0161	0.123	14.62	0.446	2.60	
3	0.575	0.0187	0.107	0.539	0.0128	0.111	13.52	0.401	2.70	
4	0.580	0.0196	0.117	0.564	0.0148	0.108	13.79	0.432	2.73	
5	0.541	0.0206	0.099	0.470	0.0125	0.100	12.41	0.429	2.39	Rest.
6	0.451	0.0152	0.082	0.617	0.0158	0.160	12.16	0.370	2.61	Labor.
7	0.595	0.0144	0.117	0.528	0.0106	0.123	13.74	0.316	2.85	"
8	0.526	0.0180	0.102	0.476	0.0101	0.103	12.22	0.370	2.45	"
9	0.591	0.0182	0.100	0.499	0.0128	0.096	13.44	0.393	2.37	
10	0.531	0.0212	0.109	0.580	0.0144	0.094	13.14	0.453	2.50	
11	0.541	0.0175	0.100	0.607	0.0130	0.117	13.51	0.383	2.53	
12	0.549	0.0183	0.098	0.491	0.0099	0.101	12.72	0.372	2.38	Rest.
13	0.501	0.0158	0.091	0.529	0.0128	0.113	12.26	0.356	2.36	Increased diet.
14	0.436	0.0151	0.089	0.549	0.0126	0.111	11.36	0.342	2.31	
15	0.477	0.0190	0.099	0.0176	0.121	13.66	0.445	2.56	
16	0.421	0.0172	0.074	0.466	0.0163	0.108	10.47	0.406	2.04	Labor.
17	0.557	0.0202	0.100	0.627	0.0131	0.118	13.92	0.428	2.54	"
18	0.597	0.0233	0.091	0.447	0.0212	0.134	12.98	0.541	2.56	Labor and dancing.
Daily av.	0.535	0.0182	0.099	0.531	0.0140	0.115	12.89	0.403	2.51	

For each of the last six days the same foods were eaten, but the quantities were rearranged, and there was added :

Confectionery 100 gms.
Total fuel value 3140 calories.

Usually the subject spent his time in the laboratory, but there were two periods of hard labor and two days of rest. The days of labor were those when at least half of the day was spent in unusual out-of-door work of a severe character. On the last day he danced; in addition, until nearly midnight. The rest days were Sundays, when there was as little exertion as possible. The night period extended from 10 P. M. to 6 A. M., and the day period from 6 A. M. to 10 P. M.

Average uric acid for 6 labor days 0.405 gm.
Average uric acid for remaining 12 days 0.402 "
Average daily uric acid for 12 days of moderate diet . . 0.395 "
Average daily uric acid for 6 days of increased diet . . 0.412 "

Subject E. — This was a university student, weighing 98 kilos, with clothing. He was a member of the foot-ball squad, although not at this time in active training. The diet was purin-free, but not fixed otherwise as to kinds or amounts. It consisted of eggs, potatoes, wheat bread, "Malta Vita" (a wheat food), butter, sugar, and salad. No meat, tea, coffee, or chocolate was taken, and of the rest as much was eaten as was desired.

COMPOSITION OF THE URINE.

Day.	Nitrogen.	Uric acid.	P ₂ O ₅ .	
	grams	grams	grams	
1	17.84	0.916	2.14	
2	19.29	0.711	3.94	
3	17.28	0.845	2.70	
4	23.28	1.055	3.28	After long walk.
5	20.86	0.957	3.91	
6	16.22	0.861	2.79	
7	Urine lost	
8	14.89	0.903	2.52	
9	22.64	1.022	4.41	After long walk.
10	21.54	0.861	3.29	
Daily av.	19.32	0.813	3.22	

The exercise involved in the long walks on the fourth and ninth days was exhaustive.

Subject F. — This was a male, aged six years, weight, without clothing, 18.3 kilos. The diet was purin-free, but not uniform in quantity. It consisted of the same materials as that of Subject C. Dotted lines indicate the omission of one or more days, during which urine was lost. The diet was continued throughout each series.

COMPOSITION OF THE URINE.

FIRST SERIES, FEBRUARY.			
Day.	Nitrogen.	Uric Acid.	P ₂ O ₅ .
1	<small>GRAMS</small> 4.32	<small>GRAM</small> 0.197	<small>GRAMS</small> 0.78
2	5.46	0.181	1.08
3	4.16	0.238	1.91
Daily av.	4.65	0.205	1.25
SECOND SERIES, MAY.			
1	6.04	0.262	1.17
2	7.41	0.287	1.29
3	6.70	0.236	1.27
Daily av.	6.72	0.262	1.24
THIRD SERIES, JULY.			
1	4.98	0.205	0.87
2	6.20	0.198	1.10
3	6.04	0.249	1.27
4	5.20	0.226	1.34
5	5.38	0.247	1.18
Daily av.	5.56	0.225	1.15

Subject G, First Series. — This was a male, aged twenty-two months, weighing 13 kilos, with clothing. The time of year was from February 16 to March 5. With so young a child, it was not expedient to make the diet constant as to amounts. It was composed of practically purin-free foods, — milk, wheat breakfast-food, egg, bread, and cookies, with a very little apple. For various reasons, the urine of several days during the series was lost, the time being indicated by the dotted lines. The child's health was good.

COMPOSITION OF THE URINE.

Day.	Nitrogen.	Uric acid.	P ₂ O ₅ .
	GRAMS	GRAM	GRAMS
1	3.87	0.160	0.88
2	4.32	0.176	0.93
3	3.96	0.111	0.89
4	4.39	0.184	1.09
5	4.18	0.130	1.06
6	4.03	0.168	1.02
7	4.46	0.177	1.12
8	3.97	0.171	0.92
9	4.10	0.163	1.06
10	4.96	0.101
11	3.99	0.144	0.88
12	3.34	0.140	0.88
Daily av.	4.13	0.152	1.06

Subject G, Second Series. — This extended from April 18 to May 2 of the same year as the preceding series, and the conditions were the same, except that the child was recovering from a severe attack of whooping-cough. His appetite was not as good, and he appeared more languid than when in his ordinary condition.

Day.	Nitrogen.	Uric acid.	P ₂ O ₅ .
	GRAMS	GRAM	GRAM
1	1.80	0.077	0.48
2	2.91	0.083	0.52
3	2.66	0.094	0.59
4	2.73	0.131	0.64
5	3.65	0.112	0.81
6	2.99	0.086	0.60
7	3.60	0.142	0.58
8	3.19	0.124	0.62
9	3.54	0.121	0.71
10	2.57	0.115	0.46
Daily av.	2.96	0.108	0.60

RÉSUMÉ.

A review of the experimental evidence presented will indicate to what extent the thesis of Burian and Schur is tenable. They maintain that the *endogenous* purin output is variable for different individuals, but constant in quantity for the same person. The results of six series carried out on one individual (Subject A), also two series on Subject C, at different times, are summarized on the following page.

The individual factor in the output of uric acid on a purin-free diet is obvious. It is noticeable, particularly with Subject A, that this varied far less than either the elimination of nitrogen or of phosphoric acid. This relative individual constancy is the more striking in view of the marked differences in the composition of the diets at different periods. The importance of the individual disposition in contrast with the dietetic factor is seen in the comparison between the outputs of different persons living on precisely the same diet.

SUBJECT A, AVERAGES.

Series.	Nitrogen.	Uric acid.	P ₂ O ₅ .	Time.
First . .	<small>GRAMS</small> 11.99	<small>GRAM</small> 0.308	<small>GRAMS</small> 2.41	December-January, 1903
Second . .	11.58	0.305	2.86	January-February, "
Third . .	11.15	0.315	2.21	March, "
Fourth . .	12.63	0.321	2.28	May, "
Fifth . .	12.68	0.313	2.49	July, "
Sixth . .	9.99	0.298	2.10	November, "

SUBJECT C, AVERAGES.

First . .	13.41	0.478	2.21	January, "
Second . .	13.92	0.452	2.49	March, "

Subject.	Series.	Nitrogen.	Uric acid.	P ₂ O ₅ .	
A	Second	<small>GRAMS</small> 11.78	<small>GRAM</small> 0.305	<small>GRAMS</small> 2.86	} Same diet.
B	First	11.39	0.340	2.37	
A	Third	11.15	0.315	2.21	} Same diet.
C	Second	13.92	0.452	2.49	

During each series it was endeavored to have the customary occupation maintained each day. The protocols of Subject C, first series, and Subject D show that a considerable increase in bodily exercise did not affect the uric acid output noticeably. When the exercise was excessive, as with Subject C, second series, the output might be temporarily increased; but in spite of continued hard work it gradually diminished to the normal endogenous amount. The total excretion of nitrogen was not affected. Sherman¹ has lately failed to find any increase in uric acid elimination to result from vigorous

¹ SHERMAN: *Journal of the American Chemical Society*, 1903, xxv, p. 1159.

exercise. The rôle of training or lack of training has, however, by no means been satisfactorily demonstrated in this connection. Dunlop, Paton, Stockman, and Maccadam¹ maintain that, with subjects in good physical training, labor causes no increased output of uric acid, while the contrary is true of those whose body is not well trained. Still the figures which they give in support of this contention are not convincing. In the present experiments, it is to be noted that both Subject C and Subject E were well trained, and that with each of them exhaustive labor was followed by a rise in the excreted uric acid.

With Subject A, fifth series, and with Subject D, the day and night elimination were separately determined. The hourly elimination of uric acid with both was greater by day than by night. This statement applies not only to the averages of each series, but almost invariably to the excreted uric acid of each day in the series. The results agree with those of Pfeil,² as may be seen by recalculating his figures to a day and night basis. Since the food contained no purin compounds, the rise cannot be due to their elimination. The explanation which most naturally suggests itself is that the difference is due to a variation in the rate of cellular metabolism with the resulting decomposition of the nuclein compounds.

The data regarding the endogenous output of uric acid in children (Subjects F and G) are of interest in view of the few results with strictly purin-free diets at present available.³ In proportion to body weight the amount of endogenous uric acid eliminated is about the same as in adults. Perhaps the most striking feature is the great variation between the results of the two series with Subject G, indicating that the condition of health may modify the endogenous uric acid output to a marked degree. Fuller⁴ has lately suggested that pronounced idiosyncrasies or hereditary tendencies manifest themselves in connection with the purin excretion in children.

The catalogue of statistical data on the output of endogenous uric acid in man under satisfactory experimental conditions already pub-

¹ DUNLOP, PATON, STOCKMAN, and MACCADDAM: *Journal of physiology*, 1897-98, xxii, p. 84.

² PFEIL: *Zeitschrift für physiologische Chemie*, 1903, xl, p. 1.

³ A few statistics will be found in WALKER HALL: *The Purin Bodies, etc.*, 1903, p. 118, and in FULLER: *Lancet*, 1903, ii, p. 1012.

⁴ FULLER: *Loc. cit.*

lished by Burian and Schur, and extended by Walker Hall, may be supplemented by the observations of Bonanni,¹ Mendel, Underhill, and White,² Kaufmann and Mohr,³ and Pfeil,⁴ in addition to those of the writer.

¹ BONANNI: MOLESCHOTT'S *Untersuchungen zur Naturlehre*, 1901, xvii, p. 257.

² MENDEL, UNDERHILL, and WHITE: *This journal*: 1903, vii, p. 402.

³ MOHR: *Loc. cit.*

⁴ PFEIL: *Loc. cit.*

THE RHYTHM PRODUCED IN THE RESTING HEART
OF MOLLUSCS BY THE STIMULATION OF THE
CARDIO-ACCELERATOR NERVES.

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THE question whether stimulation of the cardiac nerves produces a contraction or a series of contractions in the quiescent heart of vertebrates is as yet undecided, although the evidence so far adduced seems to show that neither the vagus nor the sympathetic is able to produce a contraction in the perfectly resting heart. By stimulation of the sympathetic nerves, Löwit² and Gaskell³ obtained rhythmical contractions in hearts (frog, tortoise) which had been brought to a standstill by the application of a small dose of muscarin to the sinus. After a stronger dose of muscarin the stimulation of the sympathetic did not produce any visible contraction, even though, according to Gaskell, the characteristic change (increase in negativity) of the electrical tension in the heart-muscle was effected. Gaskell is not sure that the standstill produced by the small dose of muscarin is really a quiescence of the whole heart, including the sinus and the great veins. He is rather inclined to the view that as long as the stimulation of the sympathetic produces a beat or a series of beats in the auricle and the ventricle, the large veins are still contracting rhythmically, and that when the rhythm of these veins is stopped by the stronger dose of the drug, the sympathetic nerve is no longer able to produce contractions in any part of the heart.⁴

V. Cyon and Hering have obtained rhythmical contractions of the quiescent mammalian heart by stimulation of the sympathetic;

¹ Research Assistant of the Carnegie Institution.

² LÖWIT: *Archiv für die gesammte Physiologie*, 1882, xxviii, p. 313.

³ GASKELL: *Journal of physiology*, 1887, viii, p. 404.

⁴ GASKELL: Schäfer's textbook of physiology, 1900, ii, p. 218.

but, according to Friedenthal,¹ the cardiac standstill with which these investigators worked was a vagus standstill produced by stimulation of the vagus nuclei in the medulla. Friedenthal holds that the initial standstill of the mammalian (dog, cat) heart observed in case the animal is killed quickly, as, for example, by a blow on the head, is due to the stimulation of the inhibitory nervous mechanism. Stimulation of the sympathetic during this quiescence produces a rhythmical series of beats. The initial standstill is not permanent; even in the absence of stimulation of the sympathetic, the heart resumes its rhythm and maintains it for some time. When the heart again becomes quiescent, the stimulation of the sympathetic is no longer able to produce a contraction.

The difficulties in the way of determining this question in the vertebrates are stated by Friedenthal (*loc. cit.*) as follows: "The question whether the stimulation of extracardiac nerves will excite a fully arrested heart to beat is difficult to answer by experimentation, because we have no means of arresting the heart, beating with spontaneous rhythm as long as it finds itself under favorable conditions, without profound injury of the muscle cells." So far as I know, the only experiments which do not seem to be open to these objections are those of Gaskell² on the heart of the toad. In these experiments the ventricle was brought to a standstill by a clamp in the auriculo-ventricular groove, the degree of tightening of the clamp being so adjusted that the stimulation of the sympathetic was unable to remove the block, though the passage of the nervous impulse to the ventricle was still possible. That the excitatory waves in the sympathetic did reach the ventricle, was determined by the characteristic change in the electrical tension of the ventricle which always accompanies the action of the sympathetic. According to Gaskell, stimulation of the sympathetic nerves never produces a contraction in the ventricle brought to a standstill in this manner, although the condition of the ventricular muscle is very nearly normal and the stimulation effects the change in the electrical tension. These experiments point to the view that the accelerator nerves in the vertebrates are not motor in the sense that they are able to produce contractions in the quiescent heart; only it might be objected that the tightening of the clamp in the auriculo-ventricular

¹ FRIEDENTHAL: Archiv für Physiologie, 1901, p. 31; Centralblatt für Physiologie, 1902, xv, p. 619.

² GASKELL: Journal of physiology, 1887, viii, p. 412.

groove sufficiently to block the muscular conduction will, in all probability, also cause a partial blocking of the nervous excitatory wave, so that the impulse reaching the ventricle is weaker than normal.

The problem has in reality not been put to the experimental test in the invertebrates. Ransom¹ and Bottazzi and Enriques² state that stimulation of the pleuro-visceral nerves (which send fibres to the heart) in the marine gasteropod *Aplysia* accelerates the rhythm of the pulsating and starts a series of beats in the resting heart; but as neither of these observers made it a special point to determine whether the whole heart was actually at rest, their results cannot be taken as conclusive.

My own observations, incidental to other studies on the invertebrate heart, include both the molluscan, the arthropod, and the tunicate heart, but only the molluscs present conditions favorable for the determination of the question. Until a very recent date, it was generally held that the tunicate heart is not provided with ganglion cells or nerves. Hunter³ has recently described nerve cells and nerve fibres in the heart of *Molgula*. The relation and physiology of these nerve fibres are not yet made out. From observations on *Salpa*, Schultze⁴ comes to the conclusion that the heart of that tunicate cannot be influenced by the nervous system, except indirectly by contractions of the body musculature and the viscera. My own observations were mainly confined to *Ciona*. The heart of this tunicate continues to beat for hours after the body has been slit open and the respiration thus stopped, or after being excised and placed in a dish of sea-water. After leaving the heart intact in the body till it becomes quiescent, stimulation of the brain with the interrupted current produces at times a series of beats, but these contractions may be due to mechanical stimulation from contraction and displacement of parts about the heart, which always accompany the stimulation of the central ganglion. It is hardly possible to isolate the heart so that it is not affected by the movements of adjacent parts, without severing possible nervous connections with the heart.

The heart in the crab and the lobster is provided with separate

¹ RANSOM: *Journal of physiology*, 1884, v. p. 261.

² BOTTAZZI and ENRIQUES: *Archives italiennes de biologie*, 1901, xxxiv, p. 111.

³ HUNTER: *Anatomischer Anzeiger*, 1902, xxi, p. 241; *Science*, 1903, xvii, No. 424.

⁴ SCHULTZE: *Jenaische Zeitschrift für Naturwissenschaft*, 1901, xxxv, p. 221.

inhibitory and accelerator nerves, but in these animals we not only meet the same difficulty as in the vertebrates, namely, not being able to arrest the heart while retaining its normal excitability and conductivity, but also the additional difficulty that the nerves die very quickly when the circulation has ceased. Exposing the cardiac nerves for stimulation causes such profuse bleeding that the heart is soon left in an empty condition, but the empty heart usually continues to beat for from twenty to thirty minutes after the nerves have ceased to respond to stimulation.

Turning to the large and varied molluscan group of invertebrates, we are confronted with the puzzling fact that some of the members of this group appear to be provided with only inhibitory nerves, others with only accelerator nerves, and still others with nerves the stimulation of which produces both inhibition and acceleration of the heart. The auricles and the ventricle of the lamellibranchs (*Mya*, *Tapes*) are apparently provided with only inhibitory nerves. Stimulation of the visceral ganglion or the reno-cardiac nerves in these molluscs arrests the pulsating heart in diastole. When the heart is quiescent, a similar stimulation may produce an initial beat of auricle and ventricle, followed by quiescence during the stimulation, and a more or less prolonged rhythm at the cessation of the stimulation. That the heart is really quiescent can be readily determined by the aid of a lens. We have therefore in these molluscs the singular condition of inhibitory nerves producing a contraction in the resting heart.

A similar condition has been described by Ransom (*loc. cit.*) for the systemic heart of *Octopus*. It is true not only of the systemic ventricle but also of the gill ventricles. Stimulation of the visceral nerves in the cephalopods (*Octopus*, *Loligo*) arrests the pulsating systemic ventricle and the gill ventricles in diastole; but when these organs are at rest, the stimulation either does not produce a contraction till its cessation or an initial beat is produced followed by diastolic standstill during the stimulation and the usual rhythm at the end of the stimulation, just as in the lamellibranchs.

The lower gasteropods are according to all evidence provided with only accelerator-cardiac nerves. This applies to the prosobranchs (*Haliotis*, *Lucapina*, *Natica*) and the tectibranchs (*Aplysia*, *Bulla*, *Pleurobranchæa*). When we come to the nudibranchiate molluscs one of the representatives studied (*Archidoris*) is provided with accelerator nerves only, just like the lower gasteropods, while the other

representative (*Triopha*) appears to have both accelerator and inhibitory nerves.

In the pulmonates (*Helix*, *Limax*), the dominant influence of the cardiac nerves is the inhibitory, but under some conditions accelerator effects can also be obtained. The ventricle of the giant slug *Ariolimax*, on the other hand, is provided with accelerator nerves only.

The innervation of the heart of gasteropod molluscs provided with accelerator nerves is illustrated in Fig. 1. Fig. 1 A represents the

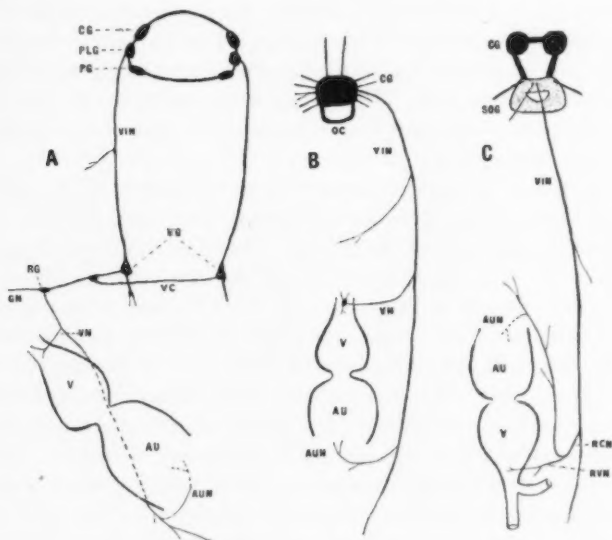


FIGURE 1.—Diagrams illustrating the innervation of the heart of gasteropods. A, *Bulla globosa*; B, *Archidoris (Montereina) nobilis*; C, *Ariolimax columbianus*. AUN, auricular nerve; AU, auricle; CG, cerebral ganglion; GN, genital nerve; OC, oesophageal commissure; PG, pedal ganglion; PLG, pleural ganglion; RCN, reno-cardiac nerve; RG, reno-genital ganglion; RVN, reno-ventricular nerve; SOG, sub-oesophageal ganglia; V, ventricle; VC, commissure between the visceral ganglia; VG, visceral ganglia; VN, ventricular nerve; VIN, visceral nerve or (in prosobranchs and tectibranchs) pleuro-visceral commissures.

visceral nervous system and its connections of the marine gasteropod *Bulla globosa*. Fibres from the common reno-cardiac nerve enter the heart both at the aortic and the auricular end. The fibres that enter by the aortic end supply the ventricle, those entering by the auricular end supply the auricle, and possibly also the ventricle to a limited

extent. In *Aplysia* and *Pleurobranchæa*, the origin and relations of the cardiac nerves are principally the same as in *Bulla*, fibres from the visceral ganglion entering the heart both by the aortic and the auricular ends. The structure and relations of the visceral nervous system of the prosobranchs differ considerably from that of the tectibranch *Bulla* or *Aplysia*, but the innervation of the heart presents no essential difference. In the prosobranchs, with two auricles (*Haliotis*, *Lucapina*), the ventricle is innervated from the visceral or "sub-anal" ganglion, the fibres entering the ventricle by the aortic end. Nerve fibres from the same ganglion also enter each auricle at its base. It is possible that the auricular nerves extend to part of the ventricle. A similar double nervous supply to the heart is also found in the prosobranchs with one auricle (*Natica*), the reno-cardiac branches taking their origin either from the visceral ganglia or from the commissure connecting the ganglia.

The relation of the cardiac nerves in the nudibranchiate molluscs is illustrated in Fig. 1 B, which represents the main visceral nerve and its cardiac branches of *Archidoris*. In these molluscs there is a tendency of the peri-oesophageal ganglia to fuse into one ganglionic mass situated dorsal to the oesophagus. In *Archidoris*, the nerve which supplies the heart takes its origin somewhat ventral on the right side of this composite ganglion or brain. A tiny branch (*vn*) connects this nerve with a small ganglion situated at the ventriculo-aortic junction, and from this ganglion nerves can be traced along the main arteries. One branch enters the ventricle. At the posterior end of the body cavity, the nerve sends a branch to the auricle (*aun*).

Fig. 1 C represents the cardiac innervation in *Ariolimax*, the giant slug of California. The main visceral nerve takes its origin from the median protuberance of the sub-oesophageal ganglion. At the level of the heart, the nerve gives off a relatively stout branch, which bifurcates, both branches entering the kidney, which in this pulmonate envelops the heart. Fibres from one of these branches can be traced to the ventriculo-aortic junction. The physiological evidence is conclusive that fibres from the reno-cardiac branch also enter the auricle at its base, but the ramifications of the nerve in the kidney and the lung sac are so minute that they cannot be followed on to the auricle. The fibres that enter the heart by the aortic end are confined to the ventricle, those entering by the auricular end to the auricle. The innervation of the heart of the snail (*Helix*) is the same as that in

Ariolimax, with the exception that the fibres entering at the base of the auricle appears to slightly influence the ventricle also.

The circulatory system of the gasteropods is partly lacunar; hence when the body cavity is opened so as to expose the heart and the visceral nerves for the purpose of experiments, the heart soon becomes empty of blood. The exposed and empty heart of the slug *Ariolimax* continues to beat rhythmically the longest of any mollusc examined, or from five to eight hours. The empty and collapsed heart of *Bulla* or *Aplysia* rarely beats for more than fifty to sixty minutes, while the exposed heart of *Haliotis* or *Archidoris* ceases to beat even sooner.

Ransom (*loc. cit.*) has called attention to the fact that in *Octopus* severance of the auricles from the systemic ventricle does not produce even a temporary cessation of the ventricular rhythm. The systemic ventricle of *Octopus* has a rhythm of its own quite independent of the auricles. This is also true for the ventricle of all the lamellibranchs and gasteropods examined by me. The normal sequence of the heart-beat is probably conditioned more by the intra-cardiac pressure and the more rapid rhythm of the auricle or auricles, than by muscular or nervous continuity between the ventricle and the auricle, from the fact that the co-ordination is quickly lost when the heart becomes empty, each part of the heart continuing to beat with its own rhythm. The standstill of the ventricle can therefore never be produced by severance of the auricle or auricles. If the ventricle of the gasteropods could have been brought to a standstill by this lesion, it would have afforded a splendid opportunity for the study of the influence of the cardiac nerves on the quiescent ventricle, when the ventricular muscle remained under practically normal conditions, because of the fact that the nerves to the ventricle enter at the aortic end. But even as it is, the gasteropod ventricle still offers a very favorable field for the study of the influence of the nerves on the resting muscle, because the empty ventricle comes to rest without the use of depressor drugs, such as muscarin, before the excitability of the muscle is greatly reduced.

In the case of the vertebrate heart, Gaskell has pointed out that if careful examination of the sinus and the great veins is not made, one may, on observing the auricle and the ventricle, be led to conclude that the heart is quiescent, while the sinus and the great veins are still beating feebly. To guard against a similar error in these experiments on the molluscan heart, the ventricle was always severed from the auricle or auricles, and, furthermore, always examined by the aid

of a lens for minute or local contractions that may have escaped the naked eye. This examination was made even when the ventricle was connected with a light recording lever for graphic registration. A horizontal position of the lever is not a sufficient evidence of the quiescence of the ventricle, because a rhythm may be (and in some instances actually was) present, too feeble to influence even the lightest recording lever.

It is furthermore necessary to guard against the possibility of the rhythm produced in the resting ventricle on stimulation of the vis-

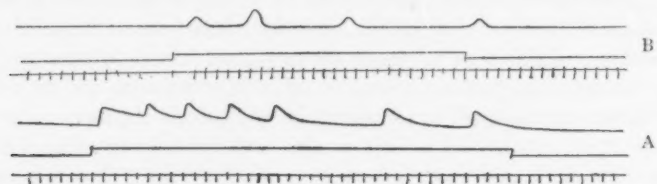


FIGURE 2. — Rhythm produced in the resting ventricle of *Haliotis* (A) and *Lucapina* (B) during stimulation of the pleuro-visceral nerves with the interrupted current. Time in seconds.

ceral or cardiac nerves being caused by variations in intracardiac pressure or by mechanical stimulation from pressure and displacement of parts adjacent to the heart. Stimulation of the visceral nerve or commissure which contains the cardiac nerves produces contraction of the gill or the lung sac, which necessarily forces some blood into the heart; but when the heart has been severed at the auriculo-ventricular junction, the ventricular rhythm cannot be ac-

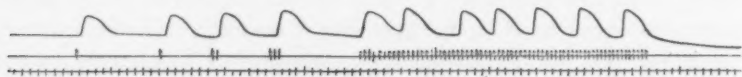


FIGURE 3. — Contractions of the resting ventricle of *Haliotis* produced by stimulating the pleuro-visceral nerves with single induced shocks. Time in seconds.

counted for in this way. It is otherwise with the tension and pressure on the ventricle from the contraction and displacement of adjacent parts. The ventricle of *Haliotis* and *Lucapina* is closely connected with the hind gut, the muscular part of which is supplied with nerves from the same ganglion as the ventricle. The heart of *Ariolimax* is imbedded in the kidney, which is contractile and supplied with motor fibres from the same nerve as the ventricle. Stimu-

lation of the visceral nerves or commissures in these molluscs produces in consequence strong contraction of the parts around the heart, but by careful dissection it is possible to isolate the ventricle, so that it is not affected by these contractions, without at the same time severing the ventricular nerves. The contractions of the quiescent ventricle following the stimulation of the visceral nerve or nerves can, under such conditions, be due to the influence of the nerve on the ventricle alone, provided that, when the electrical current is used for

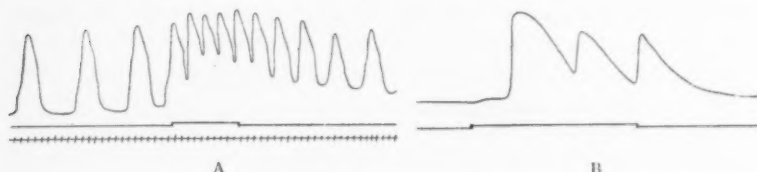


FIGURE 4. — Tracings showing the effect of stimulating the pleuro-visceral nerves on the pulsating (A) and the resting (B) ventricle of *Aplysia*. Weak interrupted current. Time in seconds.

stimulation of the nerve, there is not an escape of the current directly to the ventricle. This possible source of error must be guarded against when the cardiac nerves are stimulated between the heart and the point of union with the visceral ganglion, or the main branch of the visceral nerve, as in this case the electrodes must be placed within one to three centimetres from the ventricle. But when the pleuro-visceral commissure or the main branch of the visceral nerve is stimulated near the oesophageal ganglia, escape of the current is out of the question, for in this case the electrodes are placed at a distance of five to twelve centimetres from the ventricle.

I am therefore certain that the hearts experimented on were actually quiescent, and that the rhythm following the stimulation of the nerves was not caused by the contraction of parts adjacent to the heart. *The only alternative is that the accelerator-cardiac nerves of molluscs are able to produce rhythmical beats in the quiescent heart.*

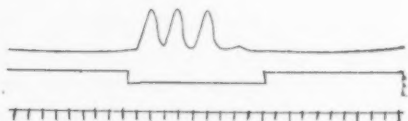


FIGURE 5. — Rhythm produced in the resting ventricle of *Archidoris* on stimulating the visceral nerve with the weak interrupted current. Time in seconds.

Typical tracings illustrating this rhythm are reproduced in Figs. 2 to 6.¹ Figs. 4 A and 6 A illustrate the influence of the stimulation of the cardiac nerves on the pulsating ventricle of *Aplysia* and *Ariolimax*, respectively. The augmentation appears both in the rate and the amplitude of contraction. As a rule, the rhythm produced in the quiescent ventricle by stimulation of the cardiac nerves does not outlast the period of stimulation, as is shown in Figs. 2, 4 A, 5, and 6 B, C. This is particularly true of the hearts of *Haliotis*, *Lucapina*, and *Archidoris* (Figs. 2 and 5). In the hearts of these animals the rhythm usually ceases after twenty to thirty seconds, even though the

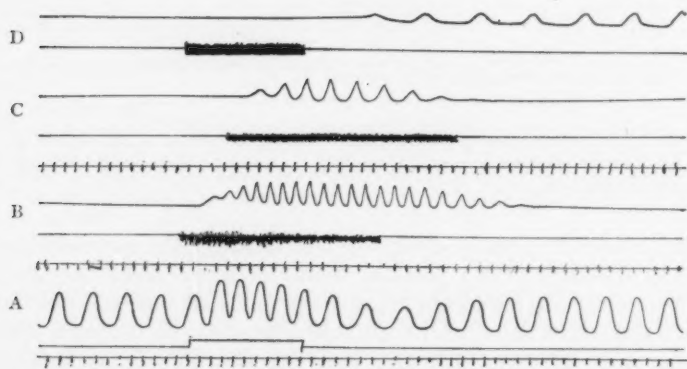


FIGURE 6.—Tracings showing the effect of stimulating the visceral nerve on the pulsating (A) and the resting (B, C, D) heart of *Ariolimax*. Weak interrupted current. Time in seconds.

stimulation of the cardiac nerve or nerves is kept up, and frequently only three or four beats at the beginning of the stimulation are produced. The more fatigued the heart, the briefer the rhythm produced by the stimulation of the nerve. In the ventricle of *Aplysia*, *Bulla*, and *Ariolimax*, the rhythm will at times outlast the period of stimulation to the extent of one-half to two minutes.

But while it is certain that the cardiac nerves of these molluscs produce beats in the resting heart, it is not plain that the molluscan

¹ In taking these tracings, the ventricle was connected with a very light recording lever by means of a silk thread secured to the auriculo-ventricular junction, or, as in the prosobranchs, by a hook made of a fine glass wire attached to the ventricle, the other end of the ventricle being fixed by securing the tissue about the aorta or the aorta itself. The tracings are to be read from left to right.

nerve-ventricle preparation presents the simple relations of an ordinary nerve-muscle preparation. A contraction of the resting ventricle, as a rule, cannot be produced by a single induced shock applied to the nerve; and the time intervening between the stimulation of the nerve and the contraction of the heart is relatively longer than would be expected in a simple muscle-nerve preparation. In *Aplysia* or *Ariolimax* a single induced shock applied to the nerve does not produce a contraction in the ventricle unless of so great intensity that the nerve is almost certain to be injured by it, in which case not a single excitatory wave but a series of impulses are produced by the stimulus. The induced shock which fails to affect the heart, when applied to the nerve singly, produces a beat or a series of beats when sent through the nerve at the rate of two to four per second. Of all the molluscs studied, the resting ventricle of *Haliotis* responds the most readily to single induced shocks applied to the pleuro-visceral nerves, each shock producing a single contraction of the ventricle. The response to the single shock is as marked in amplitude and duration as the contraction produced by a series of two or three shocks following one another in rapid succession, as will be seen from the tracing in Fig. 3. It is, of course, possible that the single induced shock produced more than one excitation wave in the nerve, or that the single excitation wave in the nerve produced a series of excitation waves in the visceral ganglion which gives rise to the cardiac nerve, so that the ventricular muscle was in reality acted on by a series of impulses.

The interval between the stimulation of the nerve and the contraction of the heart varies in the different animals. It usually exceeds one second, and may frequently be much greater, especially when the heart is relatively exhausted. In *Ariolimax* records were obtained showing a latent period of ten seconds (Fig. 6 D), but such records were exceptional. In this long latent time figure not only the latent period of the muscle, and the exceedingly slow rate of conduction of the excitatory wave in the molluscan nerve,¹ but probably also the complexity of the nervous mechanism. From experiments with nicotine on the visceral ganglion, Bottazzi and Enriques (*loc. cit.*) conclude that in *Aplysia* the cell bodies giving rise to the cardiac nerve fibres are situated in the visceral ganglion, and the neurones in the pleuro-visceral commissures make "synapses" with these cell bodies.

¹ JENKINS and CARLSON: This journal, 1903, viii, p. 251.

It is a fact that the contraction of the resting ventricle of *Aplysia* is more readily produced by the single induced shock when applied to the reno-cardiac nerve, than when applied to the pleuro-visceral commissure, that is, when applied to the cardiac nervous tract peripheral, than when applied central to the visceral ganglion.

In these molluscs the heart-muscle is supplied with truly motor nerves, not only capable of accelerating the rhythm, but also able to produce contractions of the resting muscle. The heart-muscle of the invertebrates differ considerably from the heart-muscle of the higher vertebrates. The invertebrate (molluscs, arthropods, tunicates) heart can be tetanized; it is excitable during systole, and it responds to strong stimuli with supermaximal contractions. The invertebrate heart-muscle may, therefore, be considered less differentiated than the heart-muscle in the higher vertebrates; and it is possible that with this greater differentiation is coupled the inability of the cardio-accelerator nerves to produce contractions in the resting heart. The difference between the physiological properties of the vertebrate and the invertebrate heart is, however, only relative; and I am, therefore, inclined to the view that when we shall have devised means to produce quiescence of the vertebrate heart without greatly altering the excitability and conductivity of the muscle, it will be found that the accelerator nerves are able to produce a contraction or a series of contractions just as in the molluscan heart.

SUMMARY.

Stimulation of the cardio-accelerator nerves in molluscs produces rhythmical contractions in the quiescent heart. This rhythm may outlast the period of stimulation, but usually does not.

It probably requires more than one nervous impulse to produce contraction of the resting muscle, from the fact that single induced shocks applied to the cardiac nerves do not cause a contraction.

THE NERVOUS ORIGIN OF THE HEART-BEAT IN
LIMULUS, AND THE NERVOUS NATURE OF
CO-ORDINATION OR CONDUCTION IN
THE HEART.

By A. J. CARLSON.¹

[From the Marine Biological Laboratory, Woods Hole, Mass.]

IN taking up the study of the physiology of the heart-muscle and the heart-nerves in *Limulus* in order to compare the heart of arachnids with that of crustaceans, I was rewarded by finding that in this animal the relation of the cardiac ganglia and the cardiac nerves to the heart-muscle is such that the questions of the origin of the heart-beat and the nature of the process of conduction in the heart can be settled once and for all by simple and conclusive experiments. It can now be stated as a fact that in *Limulus* the origin of the heart-beat is nervous, not muscular, and that conduction of the impulse or the co-ordination of the different parts of the heart takes place through the nerves, not through the muscular tissue.

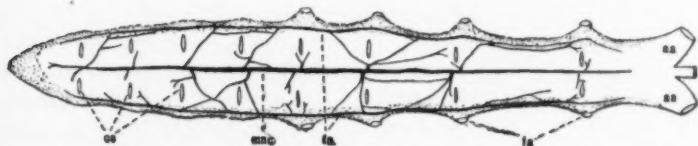


FIGURE 1.—The heart and the heart-nerves of *Limulus*, dorsal view. The heart is figured one-half the natural size of a large specimen. *aa*, anterior artery; *la* lateral arteries; *ln*, lateral nerves; *mnc*, median nerve-cord; *os*, ostia.

The structure and innervation of the *Limulus* heart are shown in Fig. 1. In large specimens the length of the heart is from fifteen to twenty centimetres. When empty and collapsed it measures about two and one-half centimetres from side to side at its widest portion. The heart is plainly segmental in its make-

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up, as indicated by the eight pairs of ostia (*os*) leading into the cavity of the heart on the dorsal side. These ostia are probably located between the segments, in which case the heart is composed of nine segments. Four pairs of arteries (*la*) are given off laterally from the corresponding four anterior segments. The main arteries (*aa*) (two, lateral and one, median) take their origin from the anterior end of the heart. No arteries are given off from the posterior end. The heart is held in position in the pericardial sinus by systems of suspensory elastic tissue fibres essentially the same as in the crab or the lobster, the suspensory ligaments being connected with the heart by means of elastic tissue fibres which run longitudinally on the surface of the heart. Below this layer of longitudinal elastic fibres is, according to Patten and Redenbaugh,¹ a homogeneous membrane (basement membrane) to which the strands of the heart-muscle are attached. The muscle bands are arranged circularly, branching and anastomosing the one with the other. The walls of the heart are thickest at the lateral angles. The muscle is of the ordinary transversely striated type like that in the heart of all arthropods (with the exception of *Peripatus*). According to Patten and Redenbaugh, there is no endothelium lining the cavity of the heart, and the blood circulates freely between the muscular strands making up the walls.

The cardiac nervous complex, confined mainly to the dorsal and lateral sides of the heart, is represented in Fig. 1. It is composed of three longitudinal nerve-trunks, one (*mnc*) in the dorso-median line, and one (*ln*) at each lateral angle, and an almost segmental system of anastomoses between the median and the lateral nerves. The median nerve is in reality a nerve-cord or elongated ganglion, as it is composed of longitudinal nerve-fibres and ganglion cells. The ganglion cells are of the bipolar type (Patten and Redenbaugh). Ganglion cells also extend for some distance into the main lateral branches of the nerve-cord. Patten and Redenbaugh state that there are no ganglion cells in the two lateral nerves. The median nerve-cord is thickest in the fourth, fifth, and sixth segments, diminishing in size both anteriorly and posteriorly. Ganglion cells are distributed throughout its whole length. The lateral nerves are also of largest size in the middle region of the heart. The median nerve is relatively large and of grayish white color, which makes it readily distinguished from the adjoining connective tissue in the living specimen. The lateral nerves are much smaller and branch considerably,

¹ PATTEN and REDENBAUGH: *Journal of morphology*, 1899, xvi, p. 91.

and are, in addition, of nearly the same color and transparency as the surrounding connective tissue ; nevertheless they can be isolated in the living heart without the aid of a lens, and without injury to the heart-muscle. Both the lateral nerves and the dorso-median nerve-cord are separated from the heart-muscle by the ectocardium or basement membrane of Patten and Redenbaugh, and can therefore, by careful dissection, be isolated for experimental purposes without the slightest injury to the heart.

The main branches from the median nerve-cord to the walls of heart and the lateral nerves are not as definitely segmental in their points of origin as would appear from the drawings of Milne-Edwards.¹ Some individual variations appear, but on the whole a pair of nerves is given off at the level of each pair of ostia, those given off in the middle region of the heart being the stoutest. These lateral branches bifurcate and anastomose extensively on the dorsal side of the heart, one or more branches joining the lateral nerves. The smaller branches are especially abundant about the ostia. In addition to these main lateral branches the median nerve-cord also gives off numerous tiny branches which penetrate the walls immediately ventral and lateral to the nerve-cord, and therefore cannot be followed for any distance. The connection of the central with the cardiac nervous system is made at various levels of the median nerve-cord. This will be considered in another place.

The vascular system is partly lacunar ; exposing the heart either from the ventral or the dorsal side leaves it, therefore, empty of blood, but, owing to the suspensory ligaments, the heart does not collapse. In the absence of blood in the pericardial space, air is sucked in through the ostia and forced through the arteries. When the heart is exposed from the dorsal side, by removing the digestive tract and part of the reproductive gland, care being taken not to sever the connections of the ventral or central with the cardiac nervous system, the rhythm of the heart is at first irregular and relatively slow, usually not exceeding twelve to sixteen beats per minute, and sometimes falling as low as eight to ten per minute. The slowness and irregularity of the rhythm is not due to the heart being empty of blood, because the irregularities disappear when the connections between the ventral and the cardiac nervous systems are severed, and they do not appear when the heart is exposed from the dorsal side, an operation which necessarily severs these connections. The rhythm of the heart ex-

¹ MILNE-EDWARDS: *Annales des sciences naturelles*, 1873, xvii, ser. 5.

posed from the dorsal side is regular from the first, the rate of pulsation varying in different individuals from eighteen to twenty-eight, the usual being about twenty beats per minute. If the hearts are protected from evaporation, this rhythm is kept up with perfect regularity for from twelve to fifteen hours, provided the animal is in good condition when prepared. Hearts from specimens in poor condition cease to pulsate much sooner.

The first thing about the heart-beat that arrests the attention of the observer is the apparently simultaneous contraction of all the parts of the heart. We have here a tubular or segmental heart half a foot in length, yet in the fresh specimens no difference in the beginning of contraction or relaxation of the foremost and the hindmost segments can be made out with the unaided eye. This is in striking contrast with the exceedingly slow propagation of the contraction wave in the tubular heart of the tunicates. The contraction of the *Limulus* heart must either begin practically at the same time in all the segments, or else the conduction of the contraction from segment to segment is much more rapid than the conduction even in the vertebrate heart. When the empty heart has been beating for several hours, or till nearly exhausted, and the rate of pulsation is in consequence much reduced, it can be made out even with the unaided eye that the contractions start in the posterior third of the heart and travel anteriorly. This is evidently also the condition in the fresh and vigorous heart. The processes which effect co-ordination in this heart are therefore conducted at a very rapid rate from one end of the heart to the other. Is it a conduction in the nerve-fibres or in the heart-muscle itself? Two simple experiments decide the question. Lesion of the median nerve-cord and the two lateral nerves in any segment of the heart destroys the co-ordination of the two ends of the heart on either side of the lesion; and, conversely, cross-section of the heart in any segment, leaving the longitudinal nerves intact, does not interfere with the co-ordination, the portions of the heart on either side of the cross-section keeping in perfect unison. As the nerves are separated from the heart-muscle by the basement membrane, every muscle-fibre can be severed in the cross-section without injury to the nerves. Whatever co-ordination or conduction that is effected between the two ends of the heart after such a lesion must, therefore, be brought about by means of the nerves alone. The abolition of co-ordination by sectioning the longitudinal nerves is immediate and permanent. Both ends of the heart continue to beat, but

with independent rhythm, the contraction not passing the region of the lesion either in the postero-anterior or in the reverse direction. There is no exception to these reactions. The experiments are so simple that any beginner in physiology can perform them. The only conclusion to be drawn from these reactions is that *conduction in the heart of this animal takes place in the nervous and does not take place in the muscular tissue.*

The lateral nerves are not essential to the co-ordination or conduction. The whole heart and the two lateral nerves may be severed, leaving only the median nerve-cord intact, yet the co-ordination of the two ends of the heart is maintained. The lateral nerves can, however, effect co-ordination to some extent, especially when a lesion of the median nerve-cord is made in the middle and posterior regions of the heart, leaving the heart walls and the lateral nerves intact. But in this case the co-ordination may also be partly due to the presence of anastomosing branches from the nerve-cord passing parallel to it to the next segment, as well as obliquely to the lateral nerves (see Fig. 1). The segmental distribution of the neurones in the nerve-cord will be considered in another connection.

From the fact that in this animal the conduction or co-ordination is concerned with nervous and not with muscular elements we may not conclude that the condition is the same in all hearts, invertebrate as well as vertebrate. Engelmann's classical "zigzag experiment" on the amphibian heart argues so strongly in favor of the view that the conduction takes place through the muscle substance that I believe the majority of physiologists to-day accept that theory. But it seems to me that the purely muscular nature of conduction is not yet an established fact for the heart of any animal, for even the great amount of work done to determine the nature of the processes of co-ordination in the vertebrate heart has not yielded a demonstration of the purely muscular nature of conduction approaching the decisiveness of the proof of the purely nervous nature of the conduction in the heart of *Limulus*.

Having found that the co-ordination of the heart is effected solely by means of the nervous elements, it was but a step to extirpate the median nerve-cord and the lateral nerves in order to determine whether without these the heart beats at all. It has already been stated that the complete removal of the median nerve-cord, together with the main lateral branches, as well as the lateral nerves, can be accomplished without the least injury to the heart, so that any effects

following the extirpation of these nervous elements cannot be ascribed to injury to the heart-muscle. The results of this line of experiments are just as conclusive as those proving the nervous nature of the co-ordination. A heart or part of a heart that will beat with perfect rhythm for from twelve to fifteen hours, when the median nerve-cord is left intact, ceases to beat immediately and permanently on extirpation of the nerve-cord. The heart or part of the heart from which the nerve-cord has been removed may be made to contract by mechanical or electrical stimulation, but the contraction always ceases with the cessation of the stimulation. I have never observed a spontaneous contraction in a heart or part of the heart deprived of the nerve-cord. The presence of the lateral nerves is not sufficient to maintain the rhythm in the absence of the median nerve-cord. And removing these nerves, leaving the median nerve-cord intact, greatly diminishes the strength and regularity of the contraction in the different segments, but the rhythm and co-ordination are still maintained. *The heart-beat in Limulus is, therefore, of purely nervous origin, the result of rhythmic nervous impulses sent out from the median nerve-cord.*

Thanks to the great length of the heart, this experiment can be varied in several ways, always yielding the same results. The heart may be cut up into four parts of approximately two segments each, each portion continuing to beat with its own independent rhythm. Sectioning or any mechanical handling of the median nerve-cord always produces acceleration, and sometimes temporary inco-ordination of the rhythm. In fatigued hearts, the initial acceleration may be followed by quiescence lasting for several minutes. The rate of pulsation is almost invariably greatest in the two portions involving the posterior end of the heart. Now the nerve-cord may be removed from either or both of these portions, and the rhythm ceases at once and for all, while the control portions, with the nerve-cord intact, continue to beat for hours. A single segment in any portion of the heart will beat rhythmically, provided the nerve-cord is intact. I have even seen localized rhythmical contractions in segments from which the main part of the nerve-cord had been removed, but one or two of the large side branches left attached to the dorsal wall. Removing these branches destroyed the rhythm.

While it is true that any segment of the heart will beat rhythmically, provided the median nerve-cord is intact, such small portions of the heart do not maintain the rhythm for as long a time as the in-

tact heart, or longer portions of the heart, and frequently no rhythm at all is exhibited by portions of the heart reduced to only one segment. This is particularly the case with the anterior heart-segments. Commencing with the fifth segment, the posterior end of the heart can be divided transversely into relatively smaller portions, and still maintain the rhythm for a considerable time.

The dorso-median nerve-cord on the heart of Limulus is, therefore, an elongated ganglion whose rhythmical activity is the direct cause of the heart rhythm. All the nerve cells having to do with the direct production of the heart-beat appear to be located in this ganglion, which fact allows this crucial experiment, so far afforded by the cardiac apparatus of no other animal. The rhythmic activity of this ganglion is not dependent on the ventral nervous system. Nor is it conditioned on the maintenance of the circulation, except so far as the irrigation and nutrition of the tissues. The heart of *Limulus* allows the determination in what way, if any, the rhythmic discharges of the nerve cells are conditioned by afferent impulses from the heart. This question will be considered in another paper.

The nerves that pass from the median nerve-cord to the heart-muscle are of the ordinary motor type. Stimulation of these nerves (the two lateral nerves and their ramifications) produces, not a rhythmical series of beats in the resting and an acceleration of the rhythm in the pulsating heart, but a tetanus closely resembling that produced in skeletal muscle on the stimulation of a motor nerve. Stimulation of the lateral nerves produces contraction in the muscle hours after the rhythm has ceased from exhaustion or extirpation of the ganglion. The action of each lateral nerve is mainly, if not solely, confined to its own side of the heart.

The heart can be inhibited by stimulation of the ventral or central nervous system, as well as by stimulation of the median nerve-cord on the heart. The effect on the heart of stimulation of the median nerve-cord with the interrupted current depends on the strength of the current and the point of the cord stimulated. For example, a strength of the interrupted current which fails to affect the heart when applied to the nerve-cord in the first segment, when applied to the nerve-cord in the fifth or sixth segment inhibits the heart posteriorly and accelerates the rhythm or causes tetanus in the part of the heart anterior to the region stimulated. This will be considered in detail in connection with the physiology of inhibition of the *Limulus* heart.

The mechanism of the cardiac rhythm in *Limulus* is, then, in every essential similar to the mechanism of respiration, both in invertebrates and vertebrates, the contraction of the muscle being brought about by rhythmical discharges from automatic nerve-centres. And yet the cardiac rhythm in *Limulus* presents such perfect resemblance to that of the crustacean and molluscan, and even that of the vertebrate heart, that were the ganglion cells and the nerves scattered all through the substance of the heart, instead of being concentrated into one median nerve-cord and two lateral nerves lying external to the heart-muscle, so that they can be handled experimentally apart from the muscle, no one could have suspected, much less proved, that the heart of *Limulus* differed from any other heart as regards the origin of the heart-beat and the mechanism of co-ordination. And does the heart of *Limulus* form an exception, or must we, on the basis of the clearly demonstrable conditions in *Limulus*, infer that similar mechanisms are operative in hearts which have not admitted of conclusive demonstrations of the neurogen or the myogen theory? I believe that the nervous origin of the heart-beat can be demonstrated in other arthropods. The segmental heart of *Peripatus* and the myriapods is provided with a median nerve on the dorsal side of the heart, similar to that in *Limulus*, and I expect that this nerve (or nerve-cord) bears a similar relation to the heart-rhythm. In the summer of 1903 I made some experiments on the heart of the large tarantula of southern California, but satisfactory results were not obtained, partly because of scarcity of material, but especially because of the delicate structure of the heart and the difficulty of isolating its nervous connection for the purpose of experiments. But suppositions are not to be accepted in lieu of demonstration; the orientation afforded by the heart of *Limulus* makes a renewed investigation of the hearts of these forms more certain of success.

It is not within the scope of this paper to review the arguments for and against the theory of the purely muscular origin of the beat of the vertebrate heart. The rhythm of the embryonic heart, prior to the appearance of nervous elements in it, appears to me the most conclusive fact in favor of that view. Nevertheless, the presence of ganglion cells in all hearts, vertebrate as well as invertebrate, in which a thorough search for them has been made, renders a mechanism of the cardiac rhythm and co-ordination similar to that in *Limulus* at least possible.

TONUS RHYTHMS IN NORMAL HUMAN MUSCLE AND IN THE GASTROCNEMIUS OF THE CAT.

By THOMAS ANDREW STOREY.

[From the Physiological Laboratories of Stanford University and the Harvard
Medical School.]

THE researches of Ioteyko¹ indicate the probability of tonus contractions in the gastrocnemius of the frog when that animal has been subjected to the influence of veratria or certain other drugs. But so far as the writer is aware, this phenomenon has not as yet been satisfactorily demonstrated in normal striated muscle, certainly not in the striated muscle of warm-blooded animals.

In the experiments herein reported, the human abductor indicis and the gastrocnemius muscle of the cat were used. They were excited to contraction by means of currents drawn from a magneto-machine.

Graphic records of the contractions of the human muscle were obtained by means of an ergograph for the index finger.²

The contractions of the gastrocnemius of the cat were recorded by a thread leading directly from the toes of the cat's foot to a horizontal recording lever. Resistance to contraction was gained by the use of a spiral spring. Ether was used for anæsthetizing. The electrodes were needles which were plunged directly into the muscular substance. Experiments were performed with the sciatic nerve intact and severed.

¹ IOTEYKO: *Études sur la contraction tonique du muscle strié et ses excitants*, Brussels, 1903.

² STOREY: This journal, 1903, viii, p. 355. The writer wishes here to express his obligation to Prof. Warren P. Lombard for the use of his ergograph in this and other researches.

MAGNETO-MACHINE AND COMMUTATOR FOR THE PERIODIC PRODUCTION OF SINGLE OR TETANIC STIMULI.¹

The magneto-machine devised for this work has no exceptional features other than it was most carefully made with reference to its insulation and freedom of movement.

The armature revolves on the same axis as does the motor which drives it.

The commutator (Figs. 1 and 2) consists of two concentric series of plugs for making desired rates and combinations of stimuli, and two revolving brushes, which make contact with metallic plates inserted on hard rubber disks. The second brush is geared directly to the axis of the revolving armature, and the first brush is geared to the second. The inner series of plugs is made up of twelve plugs and a

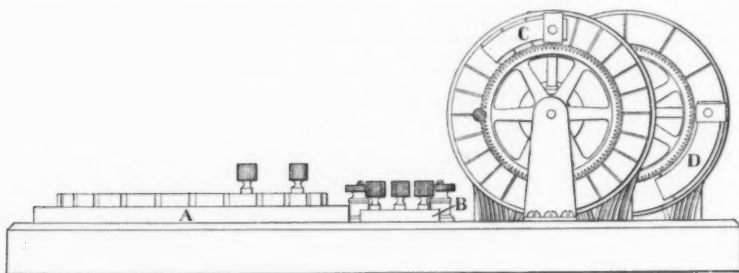


FIGURE 1. — Commutator for separating single and tetanic stimuli. Seen from one side.

brass centre plate. The centre plate is connected by wire with a brush leading from the magneto-machine. Each of the twelve plugs is connected by wire with one of the twelve plates on the disk over which the first revolving brush runs. This revolving brush is connected by wire with the inner plate of the outer series of plugs. There are twenty-four plugs in this outer series, and each one is connected by wire with one plate of the series of twenty-four over which the second revolving brush runs. This second brush is connected by wire with one of the electrodes of the ergograph. The other electrode is connected directly with the magneto-machine.

A given current then leaves the magneto-machine by one brush

¹ This apparatus was devised and constructed for the writer's use by A. O. Austin, mechanic for the Department of Physiology, Stanford University.

and passes to the central plate of the plug-board. It then passes through a plug in the inner series, and through the wire leading from that plug to its plate on the first disk. From this disk it goes to the brush as it passes over; through this brush and its wire to the outer plate of the plug-board; through any plug that may have been placed for the purpose, to the wire leading from that plug to its plate on the second disk; from here, through the second revolving brush, as it passes over that plate; and then on through the wire leading from that brush to the electrodes on the ergograph, and then back to the magneto-machine.

With the armature making three thousand revolutions a minute, there will be fifty complete revolutions a second, which will produce one hundred alternating magneto currents a second. The second

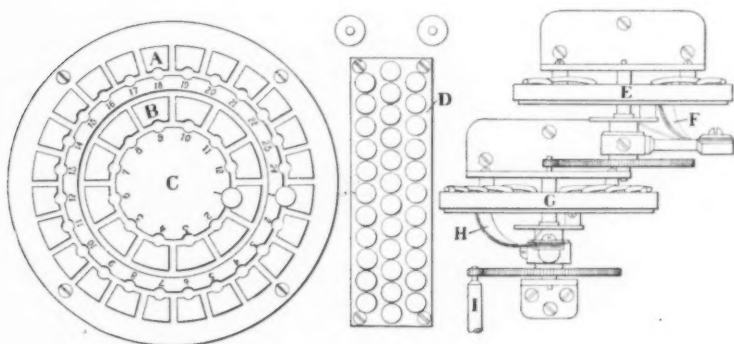


FIGURE 2. — Commutator for separating single and tetanic stimuli. Seen from above.

revolving brush is geared directly to the axis of the armature, so that it makes one complete revolution while the armature makes twelve. The brush is over one of its plates each time that a current is caused by the revolving armature. The first revolving brush is geared to the second, so that it makes one complete revolution while the second is making twelve. It is in contact with a single one of its twelve metal plates while the second revolving brush comes in contact with all of the twenty-four plates on its disk.

By properly plugging one is able by this contrivance to control within certain limitations the periodicity of and the number of currents in each excitation. With the armature revolving three thousand times a minute, one may secure single currents at intervals of

2.88, 1.44, 0.96, 0.72, 0.48, 0.24, 0.12, 0.08, 0.06, 0.04, 0.03, 0.02, and 0.01 seconds. Or each excitation may be made up of several individual currents separated by short intervals. It is possible also to control the direction of the currents that are being used. Each excitation may be caused by one or several currents going in the same direction, or the currents may be alternating.

TONUS CONTRACTIONS IN NORMAL HUMAN SKELETAL MUSCLE.

The stimulation of the human abductor indicis with single induction currents repeated once a second causes a series of quick contractions.¹ In one experiment the writer obtained six thousand six hundred records of such contractions with no variation other than a gradual diminution in the amplitude of the successive contractions.

Instead of a single exciting current repeated at regular intervals, groups of currents may be employed at regular intervals. If the groups of stimuli are used at least twice a second, slow tonus contractions may appear.

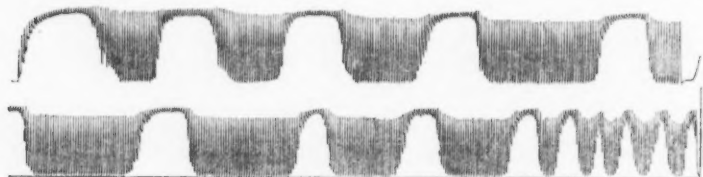


FIGURE 3. — (One-fourth the original size.) Subject, T. A. S. May 6, 1902, 3 P. M. The experiment was performed upon the left abductor indicis. The exciting currents were drawn from a magneto-machine, and the contractions were recorded by means of an ergograph for the index finger. Two groups of excitations were sent in each second. Each group was formed of two alternating currents, separated by an interval of 0.01 second. The resistance to contraction was offered by a slender rubber thread. The time-record in one-half seconds is indicated by the primary contraction records.

In Fig. 3, recorded by the human abductor indicis, both quick and tonus contractions are shown. Each of the quick contractions in this series was produced by the influence of a single group of two alternating magneto currents, 0.01 second apart. The groups were sent into the muscle regularly, at the rate of two times a second.

¹ In this paper the word "quick" will be used to distinguish the ordinary contractions of skeletal muscle from the slow tonus changes.

The quick contractions occurred then at intervals of about one-half second.¹

One may note in these tonic contractions a phase of latency, a phase of shortening, and a phase of relaxation (Table I.). The tonic contractions occurring early in a series often have in addition a phase of sustained contraction occurring between the phases of shortening and relaxation which gives the tonic myogram a flattened plateau-like crest.

TABLE I.
(Based upon Fig. 3.)

NUMBER OF TONUS CON- TRACTION.	DURATION IN SECONDS OF				HEIGHT OF CONTRAC- TION IN MM.
	Latency.	Contraction.	Relaxation.	Total contraction.	
1	13.0	8.0	21.0	39.0
2	6.5	10.0	7.0	17.0	38.5
3	7.5	11.0	5.0	16.0	38.5
4	10.5	11.0	4.0	15.0	38.0
5	19.5	9.0	3.0	12.0	37.0
6	11.0	3.5	14.5	38.0
7	18.5	6.0	4.0	10.0	37.0
8	11.5	7.5	5.0	12.5	37.0
9	13.0	5.0	3.5	8.5	37.0
10	1.5	4.0	3.0	7.0	36.0
11	1.0	2.5	1.5	4.0	34.0
12	1.5	3.0	3.0	6.0	35.0
13	1.5	3.5	2.5	6.0	35.0

In Fig. 3, the phase of shortening in the first tonic contraction begins immediately. Thirteen seconds are spent in shortening (the plateau is included) and eight seconds are spent in relaxation. After a latent period of 6.5 seconds, the phase of shortening in the second tonic contraction begins. Ten seconds are spent in that phase and

¹ The regularity of these primary contractions makes any other time record unnecessary in most of these experiments.

seven seconds in the phase of relaxation. Then follows another phase of latency preceding the third tonic contraction. The later tonic contractions in this series are accomplished with a shorter period of time for the phases of shortening and relaxation and for the interval of latency (Table I.).

TABLE II.
(Based upon the records in Fig. 6.)

FIRST GROUP.						
NUMBER OF TONUS CONTRACTIONS.	DURATION IN SECONDS OF			HEIGHT OF CONTRACTION IN MM.	SPEED IN MM. PER SECOND OF	
	Latency.	Shortening.	Relaxation.		Shortening.	Relaxation.
1	6.75	2.50	1.00	34.00	13.60	34.00
2	0.50	1.50	1.00	30.00	20.00	30.00
3	0.75	1.25	1.00	25.00	20.00	25.00
4	0.50	0.75	0.75	15.00	20.00	20.00
5	0.50	0.75	0.75	5.00	6.33	6.33
6	1.00	0.75	0.50	3.00	4.00	6.00
SECOND GROUP.						
1	3.00	1.00	0.75	29.00	29.00	38.66
2	0.25	1.00	0.50	23.00	23.00	46.00
3	0.25	0.50	0.50	15.00	30.00	30.00
4	0.50	0.50	0.50	11.00	22.00	22.00
5	0.50	1.00	0.50	12.00	12.00	24.00
6	0.50	0.75	0.50	9.00	12.00	18.00

At the end of a long series of contractions the tonic curves tend to fall into groups which resemble the fatigue curves noted in ergographic records of voluntary muscular contraction. This may be seen in Fig. 6. It will be noted that each of the last two groups recorded in Fig. 6 is made up of six tonic contractions, and that in each of these groups the height of the succeeding tonic contraction is less, and the speed of shortening and of relaxation is less, than in the preceding tonic contraction (Table II.).

It is possible to find a degree of excitation and a grade of resistance to contraction which will effect a decided reduction in the amplitude of the quick contractions and relatively much less reduction in the amplitude of the tonus contractions. It will be noted in Fig. 4 that while the primary contractions almost completely disappear, the tonic contractions still return in groups. Apparently the two sorts of contraction may be independently affected.



FIGURE 4. — (Eight-thirteenths the original size.) Subject, T. A. S. May 10, 1902.

The exciting currents were drawn from a magneto-machine, and the contractions were recorded by means of an ergograph for the index finger. Two groups of excitations were sent in to the left abductor indicis muscle each second. Each group was formed of 17 alternating currents. A spiral spring gave a resistance of 8 ounces at the abscissa and 22 ounces at the summit of the highest contraction record.

Macroscopically the quick contractions are evidently affected by the tonus contractions chiefly during the phase of relaxation. This fact is more evident in Fig. 5, which was taken from a record made on a slowly moving drum. In every instance during the tonus contraction, the relaxation of the superimposed quick contraction is retarded. Later in the experiment this influence upon the descending limb of the quick contraction is the only evidence of the presence of the tonus contraction.

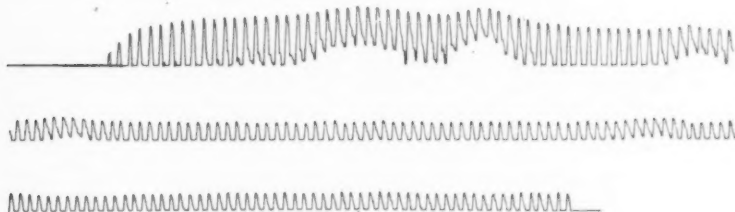


FIGURE 5. — (Four-sevenths the original size.) Subject, T. A. S. May 6, 1902. Six alternating currents formed each exciting group. A spiral spring was used for resistance. The other conditions were the same as in the preceding experiment. Only portions of the original record are reproduced here.

The figures in this paper showing records made by human muscle were all reproduced from records secured in experiments made by the writer upon himself. Similar records have been obtained from

other individuals under more or less similar conditions. Experience indicates that the strength of the excitation, the number of currents in each exciting group, the rate of excitation, and the degree of

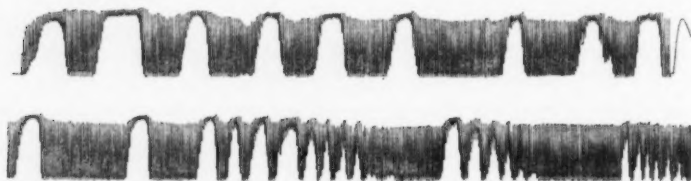


FIGURE 6. — (One-fourth the original size.) Subject, T. A. S. May 6, 1902. Conditions the same as those described under Figure 3.

resistance to be overcome by contraction must be adjusted for each individual. In addition the individual experimented upon must be able to eliminate central nervous impulses from participation in the experiment. There is no certainty in any given case that this has

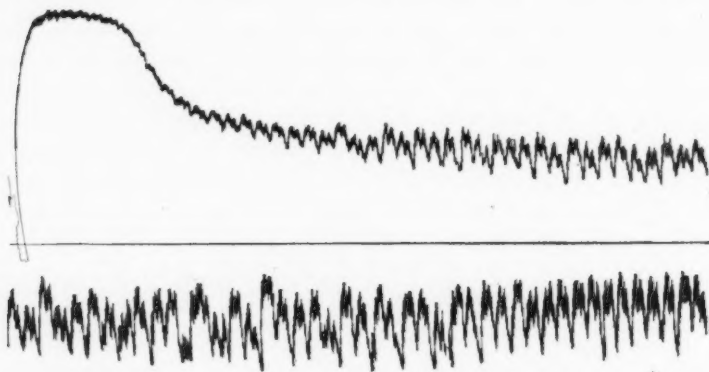


FIGURE 7. — (Two-thirds the original size.) Gastrocnemius of the cat. July 13, 1903. The exciting currents were taken from a magneto-machine, and the records were recorded by means of an ordinary recording device. The excitations were made in groups, four times a second. Each group was formed of several very strong alternating currents. The contractions were made against the resistance of a light spiral spring.

been accomplished. The writer has secured typical curves from his own muscle while engaged in mathematical computation and while reading from a book.

That these contractions are of peripheral origin is further indicated

by the experiments mentioned below, showing that analogous contractions may, under like conditions, be obtained from the gastrocnemius of the cat when the sciatic nerve is severed.

TONIC CONTRACTION OF THE GASTROCNEMIUS OF THE CAT.

Upon subjecting the gastrocnemius muscle of the cat to conditions similar to those described above, records may be secured which resemble those made by the human muscle (Fig. 7). The intervals between the tonic contractions here are considerably less than those occurring in human muscle and the tonic contractions have not so far tended to fall into groups as do those from the human muscle.

The characteristics of these contractions are the same when produced while the sciatic nerve is intact or when it is severed. Evidently then the tonus contractions are of peripheral origin.

With stronger excitations the quick contractions are reduced in amplitude and the tonus contractions become more prominent. Figs. 7 and 8 illustrate the influence of stronger excitation. Fig. 9 shows the effect of a milder excitation.

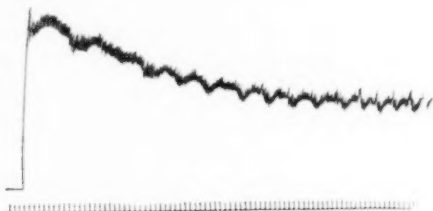


FIGURE 8.—Gastrocnemius of the cat. July 12, 1903. Exciting currents were taken from the secondary coil of an inductorium. The primary current was drawn from six Daniell cells. The hammer on the primary coil was vibrating about 50 times a second. A metronome vibrating four times a second was placed in the secondary circuit. Each swing of the metronome immersed a platinum point in a cup of mercury, thus closing the circuit long enough for the passage of several secondary currents (probably six or eight). The secondary coil stood at 0.

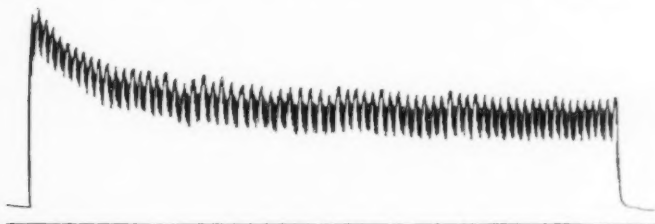


FIGURE 9.—The conditions in this experiment were the same as those described in Figure 6. There were not so many currents in each exciting group (probably four or six).

CONCLUSIONS.

The figures in this paper show the presence of tonus contractions in normal striated muscle of the cat and the human being. In human muscle these contractions are of several seconds' duration and at the end of a long series of contractions they tend to occur in groups which resemble in their profile the fatigue curves noted in ergographic records of voluntary muscular contraction. It is possible to find a degree of excitation and a grade of resistance to contraction that will effect a decided reduction in the amplitude of the rapid contractions and relatively less reduction in the tonus contractions. Macroscopically the primary contractions are affected by the secondary principally during the phase of relaxation. In every case during the tonic contraction, the relaxation of the superimposed primary contraction was retarded. These tonic contractions are of peripheral origin; for in experimenting with the cat they occur unaltered after severing the sciatic nerve.

ON THE INTERMEDIARY METABOLISM OF THE PURIN-
BODIES: THE PRODUCTION OF ALLANTOIN
IN THE ANIMAL BODY.

BY LAFAYETTE B. MENDEL AND BENJAMIN WHITE.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

THE explanation of the genesis of uric acid in mammals which is most widely accepted at the present time assumes that it arises by some oxidative process from purin precursors either ingested (exogenous) or derived from tissue metabolism (endogenous).¹ In other types of animals, such as birds, the synthetic origin of uric acid is, of course, well established. Evidence which has been adduced in recent years tends to indicate the *possibility* of a similar formation of uric acid in man and other mammals, although the extent to which such synthetic processes take place in these organisms appears limited at the most.² Quite recently, Kutscher and Seemann³ have advocated a new theory regarding the formation of uric acid in mammals. Recalling the difficulty of oxidizing the familiar purin decomposition products of the nucleic acids in vitro to uric acid, they suggest the hypothesis that uric acid is the primary rather than the secondary product formed; that xanthin, hypoxanthin, etc., may in turn be derived readily from uric acid and thus become available for the synthesis of nuclein compounds. According to Kutscher and Seemann then, uric acid has a synthetic origin; its reduction products (purin bases) serve to build up nucleins, while any excess of the synthesized uric acid may be excreted as such, or completely oxidized in the organism.

We shall not attempt at this time to discuss the relative merits of these theories. In the light of the experimental evidence at

¹ The literature on this subject has been carefully reviewed by WIENER: *Ergebnisse der Physiologie*, 1902, i, 1, p. 555.

² The synthetic formation of uric acid in man has especially been emphasized by WIENER. See *Beiträge zur chemischen Physiologie*, 1902, ii, p. 42.

³ KUTSCHER und SEEMANN: *Zentralblatt für Physiologie*, 1903, xvii, p. 715

present available, the synthesis hypothesis scarcely seems more plausible as a satisfactory explanation of all the various phenomena of purin metabolism than the more commonly accepted one. There is no reason apparent why both modes of production may not participate in the formation of uric acid in mammals. In any event, however, uric acid will appear as an intermediary product of purin metabolism.¹ The amount of uric acid eliminated from the body is by no means a direct measure of the uric acid formed in the tissues. For it is well known that various tissues exhibit a capacity for destroying uric acid; and it has been assumed that the part appearing in the urine represents a fraction which has escaped decomposition, owing to excretion during its circulation through the kidneys.

The experimental studies of Minkowski,² Cohn,³ Salkowski,⁴ Mendel⁵ and his co-workers, as well as of subsequent investigators, have demonstrated that the introduction of purin derivatives (either as nucleic acids and their compounds, or as the free purin bases) into the organism of the dog and cat is followed by an increased excretion of uric acid and of allantoin in the urine. To these may be added the recent discovery of allantoin in the urine of the carnivorous coyote.⁶ In the urine of herbivora, allantoin has not been detected under comparable conditions;⁷ and it is likewise missed in man, although an increased elimination of uric acid may be noted in such cases.⁸ In no instance is anything like the equivalent of the purin material introduced recovered in the urine. The explanation of the qualitative and quantitative differences will depend somewhat upon the theory of intermediary purin metabolism which is accepted. But the importance of information regarding these intermediary changes

¹ Cf. BURIAN und SCHUR: *Archiv für die gesammte Physiologie*, 1901, lxxxvii, p. 353; WALKER HALL: *The purin bodies of food stuffs*, 1903, p. 108 *et seq.*

² MINKOWSKI: *Archiv für experimentelle Pathologie und Pharmakologie*, 1898, xli, p. 376.

³ COHN: *Zeitschrift für physiologische Chemie*, 1898, xxv, p. 507.

⁴ SALKOWSKI: *Centralblatt für die medicinische Wissenschaften*, 1898, p. 929.

⁵ MENDEL and BROWN: *This journal*, 1900, iii, p. 261 (cat); MENDEL and JACKSON, *Ibid.*, 1900, iv, p. 163; MENDEL, UNDERHILL, and WHITE: *Ibid.*, 1902-3, viii, p. 377 (nucleic acid).

⁶ SWAIN: Doctoral dissertation, Yale University, 1904 (unpublished).

⁷ Cf. MENDEL, UNDERHILL, and WHITE: *Loc. cit.* (rabbit); GIBSON: *This journal*, 1903, ix, p. 391 (muskrat). To these may be added unpublished observations (on the guinea-pig) made by Mr. W. W. DUKE in our laboratory.

⁸ Cf. MINKOWSKI: *Loc. cit.*, p. 398; LOEWI: *Archiv für experimentelle Pathologie und Pharmakologie*, 1900, xlv, p. 22.

is obvious from the standpoint of both physiological and pathological phenomena. If uric acid is an intermediary product which is ordinarily in large part destroyed in the body, through what stages is its decomposition accomplished? How is this process influenced by abnormal conditions? To some of these problems we have directed our attention in this paper.

The laboratory decomposition products of uric acid vary with the methods of attack employed. Three series of derivatives are well-known, viz., (1) glycocoll, carbon dioxide, and ammonia; (2) alloxan, yielding in turn oxalic acid and urea; (3) allantoin and carbon dioxide, the former further yielding urea and oxalic acid. Wiener¹ has demonstrated that in the rabbit uric acid may give rise to glycocoll in its cleavage, and he is accordingly inclined to exclude the other compounds (oxalic acid, allantoin) as metabolism products of uric acid. In some cases this explanation appears satisfactory; and accordingly the failure to discover typical metabolism products of uric acid in the urine is easily understood when we consider the various ways in which glycocoll may be disposed of in the body.

The formation of allantoin from uric acid in the animal body has already been the subject of a research by Swain² from our laboratory, and the work of the previous investigators is therein reviewed. Unlike the earlier workers, Poduschka had failed in Pohl's laboratory to obtain any allantoin in the urine after feeding uric acid to dogs. He

Uric acid fed.	Allantoin excreted.	
	Dog, 17 K.	Dog, 5 K.
grams	gram	gram
1	none	0.240
4	0.430	0.780
5	0.590	0.935
6	0.620	..
9*	0.987	..
* This was fed in the course of three days.		

¹ See his review in *Ergebnisse der Physiologie*, 1902, i, 1, p. 620, for a discussion of this subject.

² SWAIN: This journal, 1901, vi, p. 38.

fed a maximum of two grams of sodium urate to medium-sized animals. Swain verified this observation by failing likewise to find allantoin after small doses of uric acid were fed. But with larger doses allantoin was always obtained, the quantity (per unit of ingested uric acid) varying with the size of the animal, as shown in the preceding table.

It seemed reasonable to conclude from such experiments that uric acid is destroyed in the organism of the dog and cat, yielding under certain conditions allantoin in addition to urea. Salkowski¹ has arrived at similar results. The presence or absence of allantoin, and the relative quantity found after ingestion of uric acid, may be assumed to vary with the oxidizing capacity of the organism, more or less urea being formed, according to the circumstances. If allantoin is an intermediary product in the combustion of uric acid in the body, it is easy to understand how increasing quantities of it may be excreted unchanged, when the amount of its precursor (uric acid) is so great as to overtax the oxidizing capacity of the organism. Accordingly a large animal might easily break down completely a quantity of uric acid which would give rise to the elimination of incompletely decomposed products (allantoin, oxalic acid) in a smaller animal, as in Swain's experiments.

The theory of the decomposition of uric acid here outlined, which admits the possibility of its cleavage through the stage of allantoin, has been criticised by the Prague school.² It is asserted that if allantoin is a normal metabolic product, it ought to appear normally in the excretions; and that the results obtained by Swain have no application to *physiological* conditions, since allantoin was obtained by him only after feeding quantities of uric acid too large to have any significance in normal functions. Instead of deriving the allantoin from the uric acid, Pohl prefers to attribute its production to a secondary reaction involving autolytic changes in the liver, as in the well-known case of allantoin excretion after diamid (hydrazine) poisoning.³ A further objection which has been offered against the assumption of allantoin as a stage in uric acid decomposition in the body lies in the failure to demonstrate any simultaneous production of oxalic acid, which

¹ SALKOWSKI: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 507.

² Cf. WIENER: Ergebnisse der Physiologie, 1902, i, 1, p. 626-627, and POHL: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 368.

³ Cf. BORISSOW: Zeitschrift für physiologische Chemie, 1894, xix, p. 499; POHL: *Loc. cit.*

in turn is an intermediate oxidation-product of allantoïn. Luzzatto¹ has obtained an increased output of oxalic acid after feeding allantoïn to rabbits; and he points out that, in view of the ready oxidation of oxalic acid in the body, it may easily escape elimination as such, by being destroyed. To us there is no apparent reason for denying the formation of any easily oxidizable compound (such as glyccoll, allantoïn, or oxalic acid) in *intermediary* metabolism, because it happens to be missed as an end product in the excretions. Only under suitable conditions, such as by synthesis with other radicals or by escape from oxidation, do the intermediary products make their appearance.

INTRAVENOUS INJECTIONS OF URIC ACID.

Recorded experiments on the fate of uric acid introduced into the body by ways other than *per os*, are relatively few in number. Mendel, Underhill, and White² found that the introduction of nucleic acid, either intravenously, intraperitoneally, or subcutaneously, is followed by an elimination of allantoïn in the dog and cat. The subcutaneous administration of urates has been noted to induce a slight increase in the output of uric acid. Direct introduction of uric acid intravenously has given no data of value up to the present time; the oxalate of calcium occasionally detected by the earlier investigators in the urine was not estimated quantitatively, nor considered in relation to the diet.³ In the hands of Burian and Schur⁴ the injection of urates was even followed by a diminished output of urinary 'purin-bodies'. None of the investigators report allantoïn, the presence of which might reasonably be expected if it represents an intermediary product. Our own experiments in this direction have been more successful.

Methods. — The animals (dogs, cats, and rabbits) were always fed for several days before each trial on a purin-free diet consisting of milk, cracker-meal and lard, or milk alone. The injections into the jugular vein were made during A. C. E. anæsthesia; the dogs previously received morphine sulphate in doses of one cgm. per kilo of body-weight subcutaneously;

¹ LUZZATTO: *Zeitschrift für physiologische Chemie*, 1903, xxxvi, p. 542.

² MENDEL, UNDERHILL, and WHITE: *This journal*, 1902, vii, p. 397.

³ Cf. WÖHLER und FRERICHS: *Annalen der Chemie*, 1848, lxxv, p. 340; GALLOIS: *Comptes rendus de l'académie des sciences*, 1857, xlv, p. 736.

⁴ BURIAN und SCHUR: *Archiv für die gesammte Physiologie*, 1901, lxxxvii, p. 292 *et seq.*

the cats, one gm. of chloral hydrate; and the rabbits, one gm. of urethane. The uric acid was injected as lithium urate in slightly alkaline half-per cent-solution from a burette, at the rate of about 200 c.c. per hour, the fluid being kept at body-temperature. After this procedure, the wound was closed and the animal returned to a suitable cage. The urine collected therefrom, or by catheter, was evaporated to a syrup. Extracts were made from this with 95 per cent alcohol, and the evaporation residues from the latter dissolved in a little water, acidified with acetic acid, and allowed to stand for at least four weeks. Crystals of allantoin which separated were then filtered off, rapidly washed with cold alcohol and ether, dried, and weighed. They were always identified by their melting point. We are aware that such a method is open to the objection of leaving small quantities of allantoin undetected. The quantitative results are low in any event; but they have the advantage of an actual isolation of the compound sought.

In the majority of cases the animals became weak and unwell for a short time after the operation and refused food; within 48 hours, however, they usually regained their normal appearance, and frequently were used again in later trials after their complete recovery.

Protocols. — A. Injection into peripheral vessels.

I. Dog of 13.4 kilos. Injection of 200 c.c. urate solution in 60 minutes. Forty c.c. urine collected during this period yielded 0.059 gm. allantoin, m.p. 215° ; 60 c.c. collected on the following day yielded 0.16 gm., m.p. 216° .

II. Dog of 13 kilos. Injection of 200 c.c. urate solution in 59 minutes. Four hundred c.c. urine collected on the following morning yielded 0.085 gm. allantoin; the next 200 c.c. urine collected during this day contained 0.09 gm. allantoin.

III. Dog of 7.4 kilos. Injection of 200 c.c. in 54 minutes. Before the operation, the urine was free from allantoin; 300 c.c. collected on the following morning gave 0.193 gm. allantoin, m.p. 217° .

IV. Dog of 24.7 kilos. Injection like those preceding. Only traces of allantoin were recovered in the succeeding 45 hours.

V. Dog of 12.2 kilos. Similar injection in 68 minutes. No allantoin was obtained.

VI. Rabbit. Injection of 200 c.c. urate solution in 60 minutes. No allantoin was obtained.

VII. — X. Two cats each received 200 c.c. urate solution, and failed to recover. No allantoin was obtained. Negative results likewise followed the injection of 50 c.c. in a third animal; while a fourth animal, after the same dose, yielded 0.073 gm. allantoin, m.p. 218° . *This cat had received no chloral.*

In considering these data it is to be noted that the allantoin cannot be attributed directly to the diet, since the latter was purin-free for several days. In one dog (I) allantoin was found in small amount by our method in the urine before the end of the injection. Absolutely negative results with dogs were observed in one case (V) only; the negative result with the rabbit corresponds with other failures to demonstrate allantoin elimination in herbivora, as already stated. It is of interest to note that the only successful outcome on cats was obtained when no narcotic was used. The possible interference of profound narcosis with oxidative or other metabolic processes will be referred to in later experiments in this paper.

It is generally admitted that the liver stands foremost among the organs in which destruction of uric acid takes place. We therefore next attempted to conduct the uric acid solutions more directly to that organ, by introducing them into the portal circulation. Pohl¹ has since described the production of allantoin by autolysis in the liver; and Blum² has found that cystin is destroyed more easily when introduced into a mesenteric vein, than when injected into a peripheral vessel.

Methods. — The operations were made on dogs. The spleen was brought to the exterior through an opening to the left of the linea alba; it was packed in absorbent cotton and continually bathed with warm saline. The injections of half-per-cent lithium urate solution were then made into an exposed splenic vein at a low pressure during 60 to 70 minutes. The splenic vessels were then ligated, the organ excised, and the abdominal wound closed with sutures. The animals recovered within 48 hours. That the splenectomy is without influence on purin metabolism has been noted by Mendel and Jackson.³ The animals were fed on a purin-free diet as in the preceding series.

Protocols. — B. Injection into the portal circulation.

I. Dog of 16.4 kilos. Injection of 200 c.c. solution. Within 5 hours 0.2 gm. allantoin was recovered from the urine. M.p. 217°.

II. Dog of 11.1 kilos. Under conditions similar to those in Experiment I (B), 0.202 gm. allantoin was obtained.

III. Same dog as in Experiment III (A). Injection of 200 c.c. urate solution yielded 0.109 gm. allantoin within 20 hours.

¹ POHL: *Archiv für experimentelle Pathologie und Pharmakologie*, 1902, xlviii, p. 372.

² BLUM: *Beiträge zur chemischen Physiologie*, 1903, v, p. 11.

³ MENDEL and JACKSON: *This journal*, 1900, iv, p. 163.

IV. Same dog as in Experiment II (A). Injection of 100 c.c., yielded an unweighed sediment of crystals of allantoin.

V. The dog which yielded no allantoin in Experiment V (A), or just before this operation, excreted 0.289 gm. allantoin (m.p. 215°) after injection of 200 c.c. urate solution.

The output of allantoin when uric acid is thus directly carried to the liver is, if anything, somewhat increased, in contrast with the results obtained after introduction into a peripheral systemic vessel. This is particularly noticeable in contrasting the two trials on the same animal (V). It was deemed advisable next to determine to what extent, if at all, the injection itself might be responsible for allantoin formation in a pathological way. Control trials were accordingly made by injecting lithium chloride solutions under comparable conditions.

Protocols. — C. Control injections.

The dogs varied in weight from 9 to 14 kilos. The quantity of lithium chloride in the 200 c.c. introduced varied from 0.2 to 0.4 gm. Three trials were made into the *jugular* vein, after one of which an unweighed sediment of microscopic allantoin crystals was found. In two animals, including the one just referred to, injection of the control solution into the *splenic* vein was without positive results; another furnished about 0.035 gm. allantoin; while a fourth dog gave 0.24 gm. allantoin in his urine. The last animal had been starved for 3 days before the operation.

These control trials were by no means absolutely negative in respect to the possibility of a "pathological" allantoin formation under the conditions of experiment. If the starving animal be omitted, the remaining dogs yielded small outputs only in one or two cases — in marked contrast with the observations made when uric acid is injected. It is worthy of note that in the one case in which 0.24 gm. allantoin was obtained, the injection was made into the portal circulation. In such instances, at least, the allantoin must be of endogenous origin, as it is after the administration of liver poisons like hydrazine sulphate, hydroxylamine, amido-guanidine, and semicarbazide hydrochloride.¹ Our attempts to isolate allantoin from the liver by Wiener's method, after urate injections, have failed in three trials.

¹ Cf. POHL: *Loc. cit.*, p. 367.

THE INFLUENCE OF DRUGS ON ALLANTOÏN EXCRETION.

In view of the important rôle of the liver in intermediary metabolism it seemed desirable to ascertain the changes which interference with the functions of that organ might bring about. Mendel and Jackson¹ have already shown that after hepatic degeneration induced by phosphorus poisoning the capacity to elaborate uric acid during a diet rich in purin bodies is not lost in the dog. Paton and Eason² have attempted to interfere with hepatic activity by administration of sulphonal. With this drug they have found that the proportion of waste nitrogen elaborated into urea is diminished; and since the formation of urea is confined chiefly to the liver, they propose to estimate interference with hepatic metabolism by determining the relative proportion of nitrogen excreted as urea. We have studied allantoin excretion in dogs intoxicated with sulphonal and quinine. The drugs were usually administered in capsules; the other experimental details were the same as those already described in this paper.

Typical protocol. — D. Injections during sulphonal intoxication.

A dog of 12 kilos was kept on a purin-free diet. Feb. 9, 1 gm. sulphonal was fed at 4.30 P. M.; Feb. 10, 2 gms. sulphonal, the animal still appearing normal; Feb. 11, 2 gms. sulphonal. The dog now appeared sleepy, and was later given 2 gms. more of sulphonal. On the following morning the animal was very sleepy and had lost control of its limbs. An injection of 200 c.c. half-per-cent lithium urate solution was made into the jugular vein in 65 minutes. *No allantoin could be obtained from the urine of the following two days.*

Five trials were made in this way, the urate solution being introduced directly into the splenic vein in some of them. The results were practically negative with regard to allantoin elimination. In one case 0.2 gm. was found in the urine, and it was noted in this animal that the intoxication was less prolonged and not as severe as in the other experiments; in another trial traces only of allantoin were found.

We have also made a study of the influence of "hepatic" drugs on nuclein metabolism. It seems unnecessary to present the extensive

¹ MENDEL and JACKSON: *Loc. cit.*, p. 168.

² PATON and EASON: *Journal of physiology*, 1901, xxv, p. 166.

protocols of these experiments here. In general the capacity of the dogs to excrete allantoin was first ascertained during a period of feeding with pancreas; similar trials were made on the animals after they had received sulphonal in large doses. *The disappearance of allantoin from the urine after pancreas feeding during sulphonal intoxication was constantly observed.* The profound effects of the drug were shown by the presence of hæmatoporphyrin in the urine. Similar, though by no means equally constant, effects were noted several years ago after administration of very large doses of quinine to dogs.¹ In careful metabolism experiments with a fixed diet the writers have also noted an increase in the ammonia-nitrogen output of dogs during the sulphonal intoxication.

SUMMARY.

The intravenous injection of uric acid (urates) in the cat and dog, like the injection of nucleic acid salts, gives rise to an excretion of allantoin. This result was more constantly observed when the uric acid was introduced into the portal circulation than when it was injected directly into a peripheral vein. In control trials (with lithium chloride) the findings were either negative or else far less pronounced than those noted after injections of uric acid. The observations after injections in the rabbit were negative with regard to allantoin excretion, as were feeding experiments with purin bodies.

The excretion (and probably the formation) of allantoin is markedly interfered with by certain drugs. Dogs subjected to sulphonal intoxication excrete considerably less allantoin after urate injections than more normal animals do.

In the deranged condition induced by sulphonal the canine organism furnishes only a slight output, if any, of allantoin after the ingestion of nucleins which yield a large quantity in healthy dogs. Further evidence is afforded of the probable genetic relation of the liver to allantoin.

¹ These experiments were conducted by Mr. J. H. GOODMAN.

THE CHEMICAL COMPOSITION OF SOME GORGONIAN CORALS.¹

BY FRANK C. COOK.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

THE Gorgonian corals afford unusually satisfactory material for the study of the organic basis of this group of lower organisms in view of the ease with which the horny axial skeleton can be separated from the adherent concretions of inorganic salts. The albuminoid nature of the skeletal material has long been recognized.² Drechsel³ applied the name *gorgonin* to the dark-brown horny substance prepared from the Mediterranean coral *Gorgonia cavolinii*, and demonstrated an unusually high content of iodine in it, as much as 7.8 per cent being found in this species. The finding of leucin, tyrosin, lysin, and lysatinin (?) among the cleavage products, taken in connection with other reactions, induced Drechsel to class the so-called gorgonin among the albuminoids, in close relation to the keratins. His investigation has lately been extended in the zoölogical station at Naples by Henze,⁴ who further obtained histidin, phenylalanin, glycocoll, and other characteristic decomposition products of 'proteids from the axial substance of *G. cavolinii*. The iodine-containing compound which Drechsel described as "Jodgorgosäure," and which he assumed to be a derivative of amino-butyric acid, has likewise been shown by Henze to be formed; but it is doubtless to be classed among the aromatic compounds. Mendel⁵

¹ The corals which Mr. Cook has examined at my suggestion were obtained through Professor Verrill from the material in the Peabody Museum of this University, and also from Professor W. R. Coe, of the Sheffield Scientific School, who kindly collected specimens for me in Bermuda. We desire to record our appreciation of the co-operation of these colleagues. — LAFAYETTE B. MENDEL.

² For the earlier literature, see O. VON FÜRTH: *Vergleichende chemische Physiologie der niederen Tiere*, 1903, p. 448.

³ DRECHSEL: *Zeitschrift für Biologie*, 1896, xxxiii, p. 90.

⁴ HENZE: *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 60.

⁵ MENDEL: This journal, 1900, iv, p. 243; also Bicentennial studies in physiological chemistry, Yale University, 1901, p. 402.

determined the iodine-content of the axial skeleton of three West Indian corals: *Gorgonia acerosa*, *Gorgonia flabellum*, and *Plexaura flexuosa*, finding them considerably poorer in iodine than *Gorgonia cavolinii*. Not a trace of bromine was found. Mendel concluded as follows: "The observations afford further justification for the belief already maintained by Drechsel, that for many organisms iodine is as essential an element as is chlorine for others; and that in the absence of iodine the normal nutrition of the organism may be interfered with. Without some assumption of this nature, it is difficult to understand why organisms like the Gorgonias should store up in their horny axial skeleton an element existing only in traces in sea-water, and apparently not entering into the constitution of the true growing coenenchyma of the animal."

An examination of the inorganic composition of the Medusæ *Aurelia* and *Cyanea* by Macallum¹ has shown that the salts of their juices contain less iodine than the sea-water in which they develop and live. The traces of iodine present do not appear to be associated with proteids or compounds which can be precipitated with alcohol. In the case of marine algæ, Dr. Dean² has found a wide variation in their content of iodine, the halogen being present in organic combination. Macallum has lately maintained that the different selective power shown by marine organisms for each constituent may be explained in some cases by reference to the history of those constituents in the ocean.³ The analyses made in the present study are intended to afford additional statistical data regarding the chemical make-up and iodine-content of Gorgonias from various regions. The list of species examined is given further below.

Methods employed.—The material was prepared for analysis by carefully removing the coenenchyma parts from the horny axial skeleton, and comminuting the latter in a mill until a fairly homogenous powder which would pass through a fine-meshed sieve was obtained. The air-dry substance was analyzed, and *moisture* determined by drying a sample for six hours at 105–110° C. The *iodine* estimations were made either by the method employed by Drechsel⁴ (involving decomposition by fusion

¹ MACALLUM: Journal of physiology, 1903, xxix, p. 237.

² DEAN: In unpublished experiments conducted in this laboratory.

³ MACALLUM: *Loc. cit.*; cf. also Transactions of the Canadian Institute, 1903–1904. (The paleochemistry of the ocean.)

⁴ DRECHSEL: Zeitschrift für Biologie, 1896, xxxiii, p. 96. This method was also employed by MENDEL: *Loc. cit.*

with an alkali, precipitation of the halogens with silver, and subsequent transformation of the iodide into chloride by means of chlorine gas); or a modification of the quantitative method of L. W. Andrews¹ was applied to the fusion products. This method involves the titration of iodine with standard potassium iodate solution in the presence of a large excess of free hydrochloric acid, using chloroform as an indicator, the final reaction, being



A comparison of the two methods on the same substance gave closely agreeing results. The *sulphur* content was determined in the usual manner after fusion of 0.5 gram substance with a mixture of sodium hydrate and potassium nitrate over an alcohol lamp. *Nitrogen* was estimated by the Kjeldahl-Gunning process, oxide of mercury being added to facilitate the decomposition. The *ash* was found by incineration.

The water content of the material prepared as described varied within narrow limits, approximating 10 per cent. The exact figures representing the loss on drying at 110° C. are given below. In the table on page 98 the composition of the samples, calculated on a water-free basis, can be compared. Owing to the small quantities of substance available, the ash content could not be determined in every instance. The composition of the organic axial material calculated to a water-free and ash-free basis for a few species is therefore given in separate columns.

The figures presented throw some light upon the possible nature of the organic basis of the corals examined. The nitrogen content, referred to the ash- and water-free material, approaches that of the familiar albuminoid substances, especially when allowance is made for the probable occurrence of foreign groups such as iodine and chlorine derivatives. Henze attempted to confirm the keratin-like nature of gorgonin by estimating its sulphur content. The so-called keratins, which unquestionably include a group of heterogeneous substances, have been classed together mainly because they exhibit a greater content of sulphur easily split off by alkalies than do the ordinary proteids. Henze found 2.32 per cent of sulphur in the case of *Gorgonia cavolinii*; but he was unable to obtain cystin, the typical sulphur-containing cleavage product of the keratin substances. The sulphur content of our materials—less than 2 per cent in every case—is apparently not as high as that of the Mediterranean species. Sulphur

¹ ANDREWS: Journal of the American chemical society, 1903, xxv, p. 576.

SUMMARY OF ANALYSES OF GORGONIAN CORALS.¹

Species.	Locality.	Loss at 110° C.	Composition of water-free substance.			Ash.	Composition of water-free and ash-free substance.		
			Iodine. ²	Nitro-gen.	Sul-phur.		Iodine. ²	Nitro-gen.	Sul-phur.
<i>Gorgonia acerosa</i> (pink)	Bermuda	11.0	1.94 *	15.75	1.03	5.09	2.0	16.5	1.08
<i>Gorgonia flabellum</i> (yellow and purple)	"	10.0	1.88 *	13.76	0.78	9.21	2.1	15.0	0.85
<i>Plexaura flexuosa</i> (light brown)	"	9.0	4.95	12.88	1.31	12.40	5.6	14.5	1.47
<i>Plexaura flexuosa</i> (dark purple)	"	8.9	4.63 *	11.73	1.26	7.76	5.0	12.6	1.35
<i>Plexaura flexuosa</i> (dark purple)	West Indies	9.1	5.34	13.26	1.16	6.00	5.7	14.1	1.23
<i>Plexarella crassa</i> (gray)	Bermuda	9.7	2.48 *	13.27	1.46	12.34	2.8	14.9	1.64
<i>Eunicea rousseaui</i> (black)	"	8.6	2.17 *	13.26	1.46				
<i>Muricea muricata</i> (yellow)	"	10.6	3.94	12.30	1.53	8.43	4.3	13.3	1.63
<i>Muricea hebes</i> (brown)	Panama	9.9	0.87	10.50	1.20				
<i>Leptogorgia rigida</i> (dark violet)	"	11.8	1.55	10.30	0.96	30.00	2.0	13.5	1.24
<i>Leptogorgia virgulata</i> (yellow)	N. Carolina	10.6	1.82	8.67	0.84				
<i>Leptogorgia virgulata</i> (red)	Florida	10.1	0.42	8.30	0.92	40.97	0.6	11.7	1.29
<i>Eugorgia aurantiaca</i> (orange)	Gulf of California	11.4	1.50	7.10	0.78				

¹ The figures represent percentages.

² The figures recorded were obtained by Andrews' method.

* The iodine content of the species marked with an asterisk was also determined by Drechsel's method with following results: *Gorgonia acerosa*, 1.86; *Gorgonia flabellum*, 1.95; *Plexaura flexuosa* (dark), 4.46; *Plexarella crassa*, 2.14; *Eunicea rousseaui*, 2.3.

was readily liberated as lead-blackening sulphide by heating with an alkali. A few trials directed to obtain mercaptan by the method of Bauer¹ failed. In harmony with Professor Mendel's observations, bromine could not be detected in either skeleton or coenenchyma, and iodine likewise was missed in the latter. Finally the lack of resemblance between the skeletal substance of the Gorgonian corals and the chitin of other invertebrates is indicated by the high nitrogen content of the coral substance, as well as the failure to obtain from it any evidence of constituent carbohydrate groups so characteristic for the chitins.

¹ BAUER: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 343.

STRUCTURAL CHANGES OF OVA IN ANISOTONIC SOLUTIONS AND SAPONIN.¹

By TORALD SOLLMANN.

[From the Pharmacological Laboratory of Western Reserve University, Cleveland, Ohio.]

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INTRODUCTORY.

IN the course of some work with starfish ova, I observed that the osmotic shrinkage which these cells show when placed in strong sugar or salt solutions, is soon followed by a very considerable swelling. I have never seen the phenomenon reported, although I do not doubt that it has been incidentally observed. It seemed to me important, and worthy of further study, since it is in apparent contradiction with osmotic laws. It can only be explained by assuming that the penetration of the sugar or salt molecules sets up an extensive decomposition of large molecules into smaller, thereby raising the osmotic pressure in the ovum far above that of the surrounding medium. These decompositions are accompanied by definite morphological changes. Extending the observations, I found that other anisotonic solutions also produce characteristic alterations which point to decomposition. The phenomena vary

¹ This investigation was carried on at the Marine Biological Laboratory, Wood's Hole, Mass.

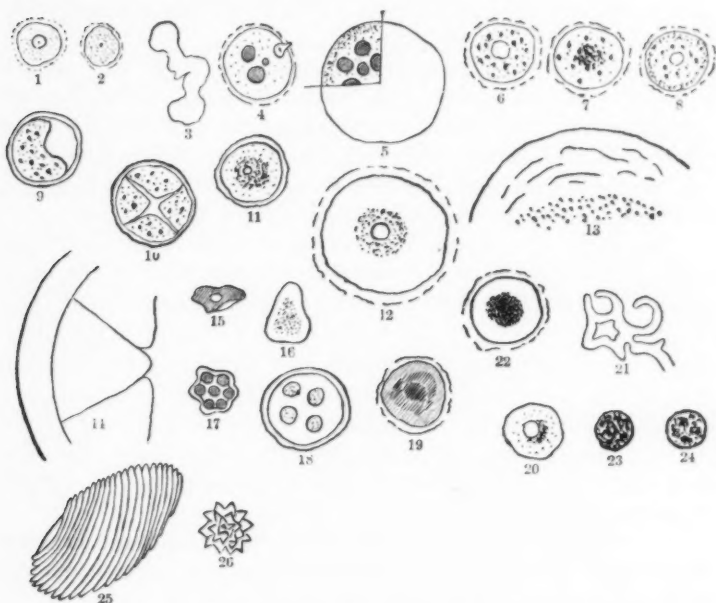
considerably in the ova of different animals, so that this method might be a valuable aid in the investigation of the structure and of the envelopes of these cells. For instance, it discovered a delicate membrane outside of the zona radiata of the *Phascolosoma ovum*, which I believe would be somewhat difficult to demonstrate by other means.

The reason why the swelling occurs in ova and not in other cells is partly to be referred to the deutoplasm, the "reserve-stuff," which is probably present in the ovum largely in insoluble form. The solution of this matter would readily account for the development of the osmotic pressure. It is quite conceivable, however, that similar decompositions occur in other cells, but that the cells do not swell because the decomposition products pass readily into the surrounding fluid. The vitelline membrane must be an essential factor in the retention of these products. Not every vitelline membrane possesses the necessary impermeability, for certain ova do not show the secondary swelling in hyperosmotic solutions. (This swelling occurs with the ova of *Asterias*, *Lumbriconereis*, *Cirratulus*, and *Phascolosoma*; it is absent in those of *Arbacia*, *Rynchobolus*, *Nereis*, and *Fundulus*.) When the decompositions have occurred, the normal structure cannot be restored by replacing the cells in normal sea-water.

I also took occasion to investigate the effect of saponin on these ova. It produced more or less marked structural changes and swelling in the ova of all the annelids which were examined, and in those of *Fundulus*, but it had almost no effect in the same concentration on those of *Asterias*, which are very sensitive to anisotonic solutions. The study of the annelids also gave me an opportunity to investigate the laking of invertebrate erythrocytes. Those of *Rynchobolus* behave precisely like vertebrate corpuscles toward all the laking agents which were applied. Those of *Phascolosoma*, however, resisted saponin and heat. Both forms are vesicular, having a distinct cuticular envelope.

SUMMARY OF PHENOMENA SHOWN BY *ASTERIAS* OVA.

1. Distilled water and dilute solutions of electrolytes dissolve a large number of the finer granules of the cytoplasm; other granules flow together to form homogeneous waxy contorted masses or globules (Fig. 3), staining selectively with eosin. These float freely



FIGURES 1 to 26. — *Asterias Ova* (LP, linear magnification of 72; HP, of 392).

1 — Normal immature ovum (LP). 2 — Matured (LP).

3 — Spongioplasmic mass, seen especially after decomposition by hyperisotonic saline solutions (HP). 4 — Ova in distilled water, dilute sea-water, $\frac{5}{32}$ *m* KCl or NaCl, and dilute $(\text{NH}_4)_2\text{SO}_4$ (LP). 5 — Ova in concentrated sea-water, hardened, and stained with methylene blue. Similar appearances are shown by the solutions mentioned under Figs. 6 to 8 (HP). 6–8 — Concentrated sea-water, 4:1* (LP); also $2\frac{1}{2}$ *m* KCl or NaCl, concentrated NH_4Cl , BaCl_2 , MgCl_2 ; and some cells in 2:1 sea-water, 1:2† sea-water, dilute Na_2HPO_4 , and spontaneous liquefaction. 9 — Fertilized ova in 4:1 sea-water (LP). 10 — Segmented ova in the same (LP). 11 — Ova in 2:1 concentrated sea-water, and in $\frac{8}{5}$ *m* NaCl or KCl (LP). 12 — Ova in dilute cane-sugar, urea, glucose, and glycerine; in dilute Na_2SO_4 , Na_2HPO_4 , and MgSO_4 ; in heat liquefaction; and on prolonged sojourn in concentrated solutions of the non-electrolytes (LP). 13 — The same (HP). 14 — Swelling and compression of blastomeres in $\frac{1}{4}$ *m* cane-sugar (HP). 15 — Shrinkage of ova in concentrated solutions (LP). 16 — Secondary clearing in concentrated solutions of non-electrolytes (LP). 17 — Segmented ova shrunk in concentrated cane-sugar (LP). 18 — Secondary swelling of the same. 19 — Dilute NH_4Cl and BaCl_2 (LP). 20 — $2\frac{1}{2}$ *m* KCl (LP). 21 — Spongioplasmic reticulum in the same (HP). 22 — Concentrated and dilute NaNO_3 , concentrated NH_4NO_3 (LP). 23–24 — Concentrated Na_2SO_4 , $(\text{NH}_4)_2\text{SO}_4$, MgSO_4 , and Na_2HPO_4 (LP). 25 — Same (HP). 26 — Dilute acid.

* Four volumes evaporated to one volume.

† One volume, diluted with distilled water to two volumes.

in a rather opaque, fluid matrix, which fills the swollen cell uniformly (Fig. 4).

2. Dilute solutions of non-electrolytes also dissolve a large number of granules. The remaining granules do not usually fuse, but arrange themselves in a compact mass around the nucleus, preserving about the normal diameter of the cell. This central mass shades off into a broad surrounding zone of a clear lymph. The size of the cells increases more than in distilled water (Fig. 12).

3. Concentrated solutions produce first a precipitation of the cytoplasm, with shrinkage, and usually with distortion of the cells. This persists with the sodium sulphate, ammonium sulphate, and sodium phosphate (Fig. 23). With the other substances which were tried, the shrinkage is followed by a secondary swelling, according to one or the other of the two following types :

4. The cells in concentrated salt-solutions show a solution of many of the granules, the cells swelling. Some large granules remain irregularly distributed through a uniform, rather opaque, fluid matrix (Fig. 6).

5. In concentrated solutions of non-electrolytes, the cells clear gradually from the margin, and then undergo the same changes as in dilute solutions (number 2, above).

6. Whilst the extremes of these different types are sharply defined, they are, nevertheless, closely related, intermediate grades being produced by certain salts, and by heat or spontaneous laking.

7. Isotonic solutions do not produce any structural changes. The effects are therefore osmotic. Fixation by formaldehyde prevents the changes completely, which makes it probable that the dissociating elements are proteids. Acids also delay the swelling, whilst alkalies favor it.

DETAILED DESCRIPTION.

The normal *Asterias* ova. — When freshly excised, the ova have the structure shown in Fig. 1. The centre is occupied by a large, clear, vesicular nucleus of 4 d.¹ This is surrounded by the densely granular cytoplasm, giving the cell a diameter of 8-8½ d. Around this is a delicate vitelline membrane, and outside of this a very faint gelatinous envelope, ¼ d. in

¹ Divisions of the eyepiece micrometer (as the instrument was lost, it is impossible for me to give the dimensions in micromillimetres. This does not detract from the comparative values, since all the measurements were made with the same instrument.)

thickness.¹ This I shall call zona radiata, for convenience, although it is not striated.

The cytoplasm and nucleolus, after fixation with formaldehyde, stain intensely with watery methylene blue. The nucleus and cytolymph, when present, do not take this stain.

This structure is preserved for some hours, until maturation. In this the cytoplasm darkens, and the vesicular portion of the nucleus disappears. The size remains unaltered (Fig. 2).

After fertilization the cell becomes surrounded by a thick fertilization membrane (Figs. 9-11).

Distilled water.—When the action is observed under high magnification, the cytoplasm is seen to become gradually clearer. Suddenly a wave passes over the cell from one side to the other: everything dissolves, except a portion of spongioplasm, which flows together in a stringy, contorted, waxy mass (Fig. 3), or into large globules. These masses stain well with methylene blue, rather selectively with eosin, poorly with osmic acid. The nucleus does not change.

Under the low power the ova have assumed the appearance of Fig. 4. The eosinophile masses float in a cloudy fluid medium. The nucleus is often eccentric, and sometimes partly extruded, as shown in the figure. The cells swell to 10-15 d. The zona radiata is also swollen, to 2 d. If these cells are replaced in sea-water they shrink to 9-10 d., but the structure remains as described. Many rupture. Fertilized ova show the same changes. The fertilization membrane also swells, to 3 d. Identical phenomena are seen in dividing ova (eight cell-stage). The blastomeres liquefy, fuse, and separate the globular masses, so that they cannot be distinguished from undivided cells. The diameter increases to 11-17 d.

Dilute sea-water.—Sea-water diluted with distilled water in the proportion of 1:1 to 1:3 produces the same phenomena as distilled water (Fig. 4). The ground substance is more transparent in the sea-water, and the swelling is perhaps not quite as great (10-13 d.). After some hours the cells again shrink somewhat (10-11 d.), very slowly, and still further (8-9 d.) when they are replaced in normal sea-water.

In the 1:1 dilution the changes occur rather more slowly; some cells escape altogether, and some resemble more those of concentrated sea-water (Figs. 6-8).

Concentrated sea-water.—Sea-water evaporated to $\frac{1}{4}$ its volume causes an immediate, but not very great shrinkage of the ova, involving the nucleus. With high magnifications, a wave of precipitation of fine granules is seen to sweep from periphery to centre. Very soon, however, a

¹ The diameter of the cell is always given exclusive of the zona radiata.

process of solution and swelling occurs, also starting from the periphery. The solution of the fine granules discloses much coarser granules, which stain brown (not black) with osmic acid, slowly with eosin, deeply with methylene blue (Fig. 5). (I cannot say whether these granules were pre-existent.)

These changes are completed in fifteen minutes.

Under the low power the ova appear as in Figs. 6-9, the cytoplasm being generally uniform through the cell, as in Fig. 6; but in some ova it is more dense around the nucleus (Fig. 7), or around the periphery (Fig. 8). The diameter varies from 9-12 d., generally 10 d. Prolonged sojourn in the solution again lessens it a trifle (9-11 d.). Transfer to normal sea-water causes a further shrinkage, but the altered structure remains.

Fertilized ova show a distinct formation of cytolymp¹, separating the denser cytoplasm from the membrane. The protoplasm is often distorted and pushed to one side, as in Fig. 9. The diameter increases to 11-13 d.

Dividing ova show the same changes (Fig. 10); the blastomeres swell, liquefy, and finally fuse.

In sea-water concentrated to $\frac{1}{2}$ its original volume, the changes are similar, but not as profound (Fig. 11). The disintegration and solution seems to involve only the outer portions of the protoplasm, so that there is an ill-defined separation into denser endoplasm and lighter exoplasm. The endoplasm stains deeply with methylene blue, the exoplasm faintly. By crushing specimens fixed with formaldehyde, a delicate vitelline membrane can be distinguished, often with adherent protoplasmic granules.

The entire process is completed in two or three minutes. The cells swell little, if at all; the endoplasm covers about 6 d.; the nuclei shrink to $2\frac{1}{2}$ d. When replaced in normal sea-water, the cells swell a trifle, and the structural changes become more pronounced, but remain of the same type.

Sea-water concentrated to $\frac{2}{3}$ its volume produces no change whatsoever.

Dilute cane-sugar solutions.—When placed in $\frac{1}{4}$ *m*² cane-sugar solution, and observed under high magnification, the cells are seen to swell gradually and to become lighter in color. The cytoplasm is also rendered very fluid, so that it flows out when the membranes are ruptured. A

¹ The term "cytolymp" is used to designate the clear watery fluid within the ovum; it consists probably of hyaloplasm, free from solid elements. The spongoplasm and metaplasm have been separated from this fluid by retraction or are partially dissolved in it.

² The letter (*m*) is used to designate a solution of one gram-molecule of the substance in a litre of water. A $\frac{1}{4}$ *m* solution contains one-fourth of this amount, etc.

great number of granules appear to dissolve, whilst others flow together and form globular and contorted masses around the nucleus, somewhat as in Fig. 3, but of finer texture. This mass also dissolves, leaving a granular material still grouped about the nucleus. The granules stain deeply with methylene blue, and fairly well with eosin and osmic acid. This central mass is surrounded by a broad sphere of limpid cytolymph, as seen in Fig. 12. This does not take the stains. The cells acquire a diameter of 14-22 d., being much larger than those in distilled water (10-15 d.). The fairly sharp separation into endoplasm and cytolymph is also a striking difference. The endoplasm retains the original dimensions of the cell (8 d.), the nucleus is swollen (4-6.5 d.), and the zona radiata is also thickened. When hardened in formaldehyde, the delicate vitelline membrane is plainly visible; it is often thrown into folds (Fig. 13). It is especially conspicuous if the cells are ruptured. These changes are far advanced in five minutes, and practically completed in fifteen minutes. No further alteration takes place in 24 hours. On transferring to *m* cane-sugar the perivitelline space contracts, the cytolymph becoming less in amount. The contraction goes on to 11 d., but the altered structure remains.

In *dividing ova* the blastomeres become disorganized and semifluid, swelling and impinging on each other, but without fusing. The fertilization membrane swells, but does not stretch much, so that it compresses the swollen blastomeres (Fig. 14).

$\frac{1}{2}$ *m* cane-sugar solution produces precisely the same effects in most lots of eggs. Sometimes, however, it is quite ineffective. In other cases, the changes are intermediate, the cytolymph remaining somewhat granular, and the swelling being less. In some cells, a layer of the denser cytoplasm remains adherent to the vitelline membrane.

Strong cane-sugar solutions. — 1.5 *m* or 2 *m* cane-sugar solutions cause at first a strong shrinking and contortion of the cell (Fig. 15), involving the nucleus, and considerably stronger than that seen in concentrated sea-water. The cytoplasm becomes darker. In a very short time, the cells begin to clear and swell at the periphery (Fig. 16). Quite suddenly solution sets in, from the periphery, and the cell swells and separates the cytolymph sphere, just as in dilute cane-sugar solutions (Fig. 12); the swelling is not, however, quite as extensive (11-13 d.). The zona radiata has also swollen (to 1 d.). The changes are completed within 10 minutes. On transferring the ova to *m* cane-sugar solution, they at first undergo a further swelling to 11-17 d., mainly by the further formation of cytolymph. The endoplasm, however, also swells to 10 d. The nucleus does not swell, nor does the structure alter. After half an hour or longer, the cytolymph has again diminished, the cell contracting to 10-15 d., at which it remains.

Mature and fertilized ova show the same changes. *Dividing ova* show at first a contraction of all the blastomeres, and a corresponding contraction of the fertilization membrane, which is therefore distorted (Fig. 17). The further changes, which occur rather late, correspond to those of the undivided ova (Fig. 18).

Isotonic (m) cane-sugar solution produces practically no change. This shows that the structural changes are not produced by the cane-sugar, but are osmotic. In accordance with this, mixtures of sea-water and *m* cane-sugar solution are also without effect.

Other non-electrolytes — glucose, urea, or glycerine — produce the same effects as cane-sugar in hyperisotonic, isotonic, and hypoisotonic solutions. Only with concentrated urea solutions, if the action is prolonged, the vitelline membrane also seems to dissolve, so that the endoplasm, swollen to 12 d., appears as faint ghosts.

Solutions of the chlorides of sodium, potassium, or ammonium. — *In $\frac{5}{32}$ m solution*, the chlorides of sodium or potassium produce practically the same effects as the corresponding dilution of sea-water (1:3) (Fig. 4); the zona radiata is rather more swollen (2½ d.).

In $\frac{5}{16}$ m solution, the decomposition is less complete than in the corresponding (1:1) sea-water, resembling more the Fig. 11. The endoplasm measures 8 d., the cell to 14 d.

In dilute ammonium chloride, the decomposition appears to be more complete, so that the spongioplasmic masses are also dissolved. There is consequently no separation of cytolymph, but the whole cell presents a cloudy appearance (Fig. 19). In many ova the vitelline membrane is also dissolved, the faint, granular protoplasm occurring as loose detritus.

Strong solutions of these chlorides act like the concentrated sea-water, a denser endoplasmic zone grading off into a lighter exoplasm (Fig. 7). The endoplasm is rather darker than in the sea-water, as if it were precipitated. In the case of potassium chloride the endoplasm tends to assume the form of a demilune, applied to the nucleus (Fig. 20). With this solution (2½ *m*) it may be seen, under high magnification, that the cytoplasm at first becomes more granular, and retracts, often separating at one place from the vitelline membrane. Then solution occurs, leaving some granules and a coarse spongioplasmic reticulum (Fig. 21) diffused through the cell, or more commonly condensed about the nucleus. During this solution the cell swells.

Chlorides of magnesium, calcium, and barium. — *In concentrated solutions* these act just like concentrated sea-water (Fig. 7).

In dilute solution, the calcium has the typical action of sea-water (Fig. 4); magnesium produces a structure more like sugar (Fig. 12), but with less swelling (11 d.); barium resembles ammonium chloride (Fig. 19).

Nitrates of sodium and ammonium.—The *concentrated solutions* produce precipitation, with very dark appearance of the cytoplasm, and but little contraction or distortion. A clear cytolymph collects, and forms a well-defined sphere about the endoplasm (Fig. 22). The appearance reminds strongly of that produced by cane-sugar (Fig. 12); it differs by the darker appearance of the endoplasm and the smaller diameter of the cell. This is 13 d. with the sodium nitrate. With the ammonium nitrate, the cytolymph is very scanty, often on only one side. This difference is seen equally strikingly with dilute solutions. The sodium nitrate corresponds exactly to the strong solution (Fig. 22), whilst the ammonium nitrate produced only darkening, and no cytolymph. Strong sodium nitrate has a similar effect on dividing ova, the cytoplasm of the blastomeres being precipitated, whilst the interstitial fluid is increased.

Sulphates of sodium, ammonium, and magnesium, and phosphate of sodium.—*Concentrated solutions* produce precipitation, darkening, and a slight shrinkage (Fig. 23), but no secondary swelling. A few cells show large dark granules (Fig. 24), which are also seen in blastomeres of dividing ova.

Under high magnification, the concentrated sodium sulphate is seen to throw the surface into parallel folds in the course of the contraction. (Fig. 25).

Dilute solutions. Some cells usually resemble those of sugar (Fig. 12), others those of water (Fig. 4). Sodium sulphate and phosphate are more of the sugar type, the cells being swollen to 10–17 d.

Magnesium sulphate is of the same type, but the swelling only reaches 14 d. Ammonium sulphate approaches nearer to the water type; almost all the cells rupture. A few cells in the sodium phosphate are more like concentrated sea-water (Fig. 6), with a diameter of 13 d.

Heat liquefaction.—Careful heating causes the same phenomena as anisotonic solutions of non-electrolytes (Fig. 12), the cells swelling to 10–11 d. When these cells are placed in distilled water, they swell like normal cells, but preserve the sugar-structure instead of assuming that of Fig. 4.

Spontaneous liquefaction. When unfertilized ova are left for a long time in sea-water, they degenerate, according to a variety of types. Some disintegrate completely. Those in which the vitelline membrane is preserved show the closest resemblance to concentrated sea-water (Figs. 6–8), the cytoplasm consisting of a rather opaque matrix with fine or coarse granules, or with the spongioplasmic masses of Fig. 4. The ova swell to 8–13 d.

Influence of various conditions on the reactions of *Asterias* ova.—*Individuality.* It can be easily observed that there is some degree of difference in the resistance of different lots of eggs, and even of the ova

of the same animal. This is more conspicuous during the early stages of the action, and with the milder reagents.

Maturation, fertilization, and segmentation do not modify the phenomena materially.

Acids. Dilute acid tends to prevent the solution, disintegration, and swelling. When laid in 4 parts hydrochloric acid to 1000 parts of sea-water, the ova darken, without change of size. Maturation and spontaneous liquefaction are retarded. Transferred to 2 *m* cane-sugar solution, the cells shrivel and assume a stellate appearance (Fig. 26). The secondary swelling (Fig. 12) is delayed over an hour, whilst it occurs in 40 minutes in sea-water. A similar retardation is observable for the swelling in dilute sugar solution.

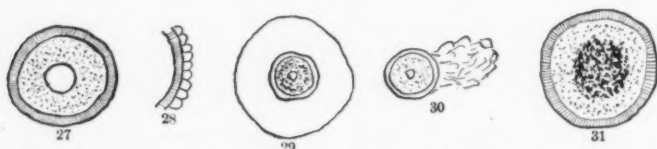
Alkalies. These hasten the corresponding processes. The cytoplasm becomes lighter, without change of size. In cane-sugar, the swelling occurs more promptly. Spontaneous liquefaction is also hastened.

Fixation of the protoplasm by formaldehyde prevents the changes completely.

Saponin (1 part quillaja saponin, Merck, in 1000 parts sea-water) has no effect on the reactions. It seems to hasten fertilization; division proceeds as in normal ova.

OVA OF PHASCOLOSOMA GOULDII.

Summary of the phenomena. — Anisotonic solutions of non-electrolytes, and the action of saponin, cause the formation of clear cytolymph, which forms a vesicular shell *outside* of the zona radiata. Electrolytes do not produce this action (except a very slight degree in dilute solutions).



FIGURES 27 to 31. — Ova of *Phascolosoma gouldii*. 27 — Normal ovum (HP). 28 — Beginning extrusion of cytolymph (HP). 29 — Completed vesicle (LP). 30 — The same with membrane ruptured (LP). 31 — Early effects of saponin.

Detailed description. — *The normal ovum* is shown in Fig. 27 under high magnification. At the centre is the large vesicular nucleus. This is surrounded by the granular cytoplasm, and this by a plainly striated zona radiata. The behavior to reagents shows that the zona radiata is covered externally by a delicate membrane. The diameter of the ova, under the low power, is 8 to 10 d.

Effects of hypoisotonic ($\frac{1}{3}$ m) cane-sugar solution.—The cytoplasm clears somewhat, but its structure does not appear greatly altered. The principal changes occur outside of the zona radiata, and very quickly.

Limpid pearly drops appear on the entire surface of the ovum (Fig. 28). These increase in size, fuse, form a continuous zone, and continue to swell until the ovum has increased to 23 d. (Fig. 29). The original cell, bounded by the zona radiata, does not alter its size. The striations of the zona radiata are lost, but reappear if the vesicle is ruptured. The vesicle is bounded externally by a delicate membrane, which is easily broken by pressure, or by osmotic changes. If the cells are replaced in sea-water, the vesicle shrinks and wrinkles, and becomes less well defined. Finally nothing is left of it but a small folded mass, the vestige of the collapsed and ruptured membrane (Fig. 30). The striation of the zona radiata reappears. If these cells are again placed in the dilute sugar solution, the vesicle does not reappear, since the membrane is broken.

The dilute sugar solution evidently sets up decompositions in the cytoplasm, which raise its osmotic pressure, with the formation of cytolymph. The zona radiata seems to be incapable of stretching. The cytolymph is therefore squeezed through the pores of the zona radiata, lifting the outer membranes as small blisters; as more cytolymph is poured out, the membrane is lifted up entirely, forming a continuous vesicle around the cell. The pressure in this vesicle obliterates the striation of the zona radiata.

Effects of hyperisotonic (2 m) cane-sugar solution.—The cells shrink at first and become distorted. The zona radiata participates in this process. The ovum then resumes its original size, and undergoes the same changes as in the sugar solution (Figs. 28 and 29). The swelling, however, reaches only 18 d.

m cane-sugar solution slowly produces the first stages of this process (Fig. 28), but does not complete it to the stage shown in Fig. 29.

Urea, in dilute and concentrated solutions, causes the same changes as the sugar.

Distilled water, diluted sea-water (1 : 4), and dilute solutions of sodium nitrate and sulphate, and of ammonium sulphate, produce some clearing and swelling of the cytoplasm (to 12 d.), but most cells do not form a vesicle. The striation of the zona radiata is preserved. A few cells produce an imperfect vesicle, especially in *dilute potassium chloride*. When cells which have failed to form a vesicle in the dilute salt solutions are transferred to *m* cane-sugar solution, the vesicle appears at once. It would therefore seem that the sugar produces a more profound decomposition, as is the case in the *Asterias* ova.

Heating causes precipitation of the cytoplasm, with some vacuolization; but it does not produce the vesicle.

Concentrated solutions of salts (4 : 1 sea-water, sodium nitrate or sul-

phate, potassium chloride, and ammonium sulphate) cause precipitation, without distortion, swelling, or vesicle formation (either the membrane is perfectly permeable to the decomposition products, or, which is more likely, the precipitation prevents the decomposition).

Saponin (0.1 per cent in sea-water). The protoplasm darkens at once. The nucleus loses its sharp boundary, and fuses with the cytoplasm. This separates into a denser endoplasm and a clearer exoplasm (Fig. 31). This passes very slowly into the typical vesicle formation (Figs. 28 and 29).

OVA OF LUMBRICONEREIS.

These separate a cytolymph inside of the zona radiata and swell in hyperisotonic sugar solution. Saponin is also effective.

The normal ova are shown in Fig. 35; they consist of a dark cytoplasm and a distinct, but not striated, zona radiata. The diameter is 21 d., under the high power. The zona radiata has a thickness of $1\frac{1}{2}$ d. In



FIGURES 32 and 33.—Ova of *Lumbriconereis*. 32—Normal. 33—The same in anisotonic solutions or in saponin (HP).

$\frac{1}{4}$ m cane-sugar solution, and in distilled water, the cytoplasm is partly dissolved, separating a peripheral zone of cytolymph (Fig. 33). This is not as clear as that formed in asterias or phascolosoma ova. The cells swell to 24 d. In 1:4 diluted sea-water they present the same structure, but swell rather more (28 d.). 2 m cane-sugar solution causes the same final appearance. Saponin produces an identical effect, the cells swelling to 26 d. The endoplasm in all the reagents retains the original dimensions of the cell, or swells slightly.

OVA OF CIRRATULUS GRANDIS.

These are very opaque. They have a narrow, but distinct, non-striated zona radiata. In the various anisotonic solutions they show very much the same effects as the ova of *Lumbriconereis*.

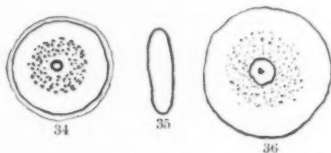
OVA OF RYNCHOBOLUS AMERICANUS.

In hypoisotonic solutions, the cytoplasm disintegrates and separates into a denser endoplasm and a very much lighter, swollen exoplasm. The latter, however, is not by far as clear as the cyto-

Structural Changes of Ova in Anisotonic Solutions. 111

lymph in the previously described varieties. The ova, further, show but a slight secondary swelling in hyperisotonic solutions and in saponin.

The normal ova (Figs. 34 and 35) are flattened. A wreath of large granules is arranged about the nucleus. These stain readily with methylene blue. The zona radiata is faint. The diameter is 19 d. In distilled water and in $\frac{1}{4}$ m cane-sugar solution, the granules dissolve, the cytoplasm becomes clearer, and separates the rather dense cytolymp (Fig. 36). The diameter increases but moderately, to 22 d. in the sugar, to 25 in the water. In 2 m cane-sugar solution, the cells shrink a trifle at first, the granules becoming lighter and more diffused. There is practically no secondary swelling. Concentrated urea solution renders the zona radiata very faint. The cells swell to 23 d. The wreath of granules remains. Saponin causes a trifling swelling. The granules fuse. The exoplasm is lighter.



FIGURES 34 to 36.—Ova of *Rhynchobolus*.
34 and 35—Normal (top and side view). 36—In hypoisotonic solutions.

OVA OF ARBACIA.

The eggs of the sea-urchin are very resistant to anisotonic solutions. They swell in dilute, and shrink in concentrated solutions. There is no secondary swelling in the latter. The structure is not noticeably altered in any of the reagents.

THE OVA OF NEREIS VIRENS

are similarly resistant, but are affected by saponin.

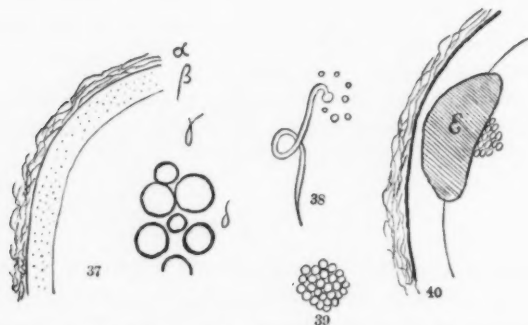
FUNDULUS OVA.

The changes, on the whole, are small.

Structure of the normal fundulus ova.—The freshly expressed ova present the appearance shown in Fig. 37. On the outside is a *zona radiata* consisting of a matted felt of hairs (α), shown in higher magnification in Fig. 38. From the effects of reagents, it would appear that these hairs are embedded in a gelatinous matrix, which is rendered adhesive by heat,

swells in hypoisotonic solutions and in urea, and shrinks in concentrated sea-water and cane-sugar; saponin appears to dissolve out this matrix, for the membrane becomes thinner and the hairs more matted. The hairs rest on a delicate *vitelline membrane*.

Within the *zona radiata* is a fairly broad, clear zone β , studded with small points, which are really the optical cross-sections of the hairs. This zone could be readily mistaken for a thick membrane. It is, however, merely a *perivitelline space*, filled with clear lymph; this becomes apparent when the yolk is made to shrink by heating, or when it is first shrunk and then dissolved by concentrated urea. It is also obliterated when the yolk swells in $\frac{1}{4}$ *m* cane-sugar or distilled water or saponin; it is narrowed in 2 *m* cane-sugar, and in concentrated ammonium sulphate. It is first widened and then obliterated in concentrated sea-water and concentrated urea.



FIGURES 37 to 40. — *Fundulus Ova*. 37 — Immature. 38 — The hairs, higher magnification. 39 — alveolar yolk structure. 40 — Blastodisc.

Within this lies the homogeneous yellow *yolk* (γ). This is contracted by heating; in concentrated urea it contracts at first, but soon dissolves and becomes almost colorless. In distilled water and concentrated sea-water or potassium chloride, its structure remains quite unchanged. In $\frac{1}{4}$ and 2 *m* cane-sugar, and in concentrated ammonium sulphate, it assumes a faint alveolar structure (Fig. 39).

A few large *oil globules* float in the yolk (Fig. 37, δ). These are not at all altered by most reagents, including saponin. In 2 *m* cane-sugar and in concentrated ammonium sulphate solution, they gradually coalesce to form one large bubble, which contains a number of stellate groups of acicular crystals, presumably of fatty acids.

The *entire ovum* shrinks and becomes folded and distorted in the 2 *m* cane-sugar and in concentrated ammonium sulphate; not in the concentrated (4:1) sea-water, nor in 2 $\frac{1}{2}$ *m* potassium chloride, nor in the

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concentrated urea solution. Changes in size in other solutions were not observed.

The formation of the blastodisc.— Within $2\frac{1}{2}$ hours (no precautions having been taken to prevent fertilization), a lenticular blastodisc (ϵ , Fig. 40) is formed in the ova kept in sea-water. This has a denser structure than the yolk. The latter assumes an alveolar structure for a short space behind the blastodisc. No further change occurs in 24 hours.

The blastodisc is also formed in distilled water, concentrated ($4:1$) sea-water, and saponin; it is delayed or prevented by $\frac{1}{4}m$ and $2m$ cane-sugar solution.

Ova in which the blastodisc is formed are more resistant to the anisotonic solutions.

The *blastodisc* is dissolved when the ova are placed in $2m$ cane-sugar, concentrated potassium chloride or urea solution, in distilled water, and by heating. It is *not affected* by $\frac{1}{4}m$ cane-sugar, concentrated sea-water, or saponin.

THE ERYTHROCYTES OF ANNELIDS.

It is well known that the body fluid of certain annelids contain a variety of red-blood cells.¹ The red pigment is "hæmerythrin." It is confined to the corpuscle.



FIGURES 41 to 46. — *Erythrocytes of Rhynchobolus*. 41 — Normal. 42 — Pseudo-amœboid crenation. 43–44 — Distortion in concentrated salt solution. 45 — Swelling in saponin. 46 — Position of the corpuscle in the field (they do not touch). 47–48. — *Erythrocytes of Phascolosoma*. 47 — Seen from the flat side. 48 — from the edge.

I encountered and studied these in *Rynchobolus americanus* and in *Phascolosoma gouldii*. In the former they are spherical (Fig. 41), in the latter discoid (Figs. 47 and 48). The *Rynchobolus* corpuscles crenate readily (Fig. 42), and may appear in this way to be amœboid. They have a fairly uniform diameter (5 d. under the high power). Both forms contain a small nucleus, staining deeply with methylene blue, and another small refracting body which does not stain (vacuole?). The body of the cell is perfectly clear, of an amber color. The cells are surrounded by a distinct cuticular

¹ A bibliography of this subject is given by R. KOBERT: *Archiv für die gesammte Physiologie*, 1903, xcviij, p. 428.

envelope. This cannot be easily distinguished in the normal cells, although its existence may be inferred from the fact that the cells never touch each other (Fig. 46). The membrane becomes plainly visible in saponin (Fig. 45). The corpuscles are not readily deformed by pressure. They do not agglutinate, and take no part in the clotting of the fluid.

The reaction of the corpuscles of Rhynchobolus to laking agents is precisely the same as that of human corpuscles.

Hyperisotonic solutions (2 m cane-sugar) causes shrivelling (Figs. 43 and 44). *Water* or $\frac{1}{4}$ m. cane-sugar causes them to swell (to 7 d.) and to lose their hæmoglobin. Complete laking also occurs in *concentrated urea solution*. After *partial drying*, they also lake in sea-water (corresponding to the reaction discovered by C. C. Guthrie¹ for human blood). In *saponin*² (1 part in 1000 of sea-water), they swell at first, and show the delicate envelope (Fig. 45). They continue to swell, and lose their hæmoglobin, the ghosts retaining the nucleus and refractive granules. The solution is finally complete, leaving only some granular débris and the nuclei.

*The reactions of the Phascolosoma corpuscles differ from the above, in that they are not affected by the same concentration of saponin. They are also not laked by heat.*³ *2 m cane-sugar solution* caused distortion. In $\frac{1}{4}$ m cane-sugar, the cells swell so as to become globular. Pressure readily causes a displacement of the nucleus, so that the contents must be fluid. Greater pressure produces wrinkling, and then discharge of the contents.

Robert (*loc. cit.*) finds that the corpuscles of the allied sipunculus nudus are also laked by the saponin Cyclamin, but that they are not agglutinated by abrin or ricin.

CONCLUSIONS.

Anisotonic solutions cause decomposition and solution of the cytoplasm of ova, raising the osmotic pressure to such a degree that the cells may swell in hyperisotonic solution.

¹ C. C. GUTHRIE: This journal, 1903, viii, p. 441.

² Crude quillaja saponin, Merck.

³ The close of my stay at Wood's Hole did not permit me to repeat these experiments, and I only quote them to call attention to the observed behavior without considering the conclusions binding.

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It is suggested that the employment of anisotonic solutions may be useful in investigating the structure and composition of the cytoplasm, and the existence and permeability of cell membranes.

The behavior of the erythrocytes of annelids toward laking agents closely resembles that of the vertebrate corpuscles.

THE EFFECT OF PARTIAL STARVATION ON THE BRAIN OF THE WHITE RAT.

By SHINKISHI HATAI.

[From the Neurological Laboratory of the University of Chicago.]

IT was the object of this investigation to determine the effect of partial starvation on the brain weight of the white rat, and also to discover how far the percentage of water and of the ether-alcohol extracts was modified under these conditions. The general conclusion which has been reached is that the form of the partial starvation here used, not only stops the growth of the brain in rats in completely grown animals, but also causes an appreciable loss in brain-substance. In such animals, however, the percentage of water and of the ether-alcohol extracts is affected to only a very slight degree.

Conditions of experiment.—White rats in the growing stage were alone used. The experimented rats were allowed to eat starch (Oswegos corn starch) in the form of pudding, beef-fat, and water, but were deprived of proteids. The animals were given an abundance of the food, starch pudding being used as the main food, while a smaller amount of fat was given frequently for a change. The experiments were continued for twenty-one days, and the body-weight was regularly determined daily before feeding. The rats for the control were given a normal diet, such as bread, milk, meat, cabbage, and other vegetables. There were two series of observation, and in such series there were six litters of animals, — each litter consisting of one group subjected to experiment, and another used for control. The control and experimented groups were kept separated. In the first six litters, both groups were kept alive for three weeks, and at the end, they were killed at the same time. In the case of the other six litters, the control group was killed at the beginning of the experiment, and the experimented group alone kept alive. The reason for this procedure will be given later.

On body-weight.—It is well known that complete starvation reduces the body-weight more rapidly than partial starvation. On

The Effect of Starvation on the Brain of the Rat. 117

the other hand, young animals are more affected than those mature. In comparing our own results with those of other investigators, this

TABLE I.
SHOWING THE BODY-WEIGHT, INITIAL AND FINAL, IN BOTH CONTROL AND EXPERIMENTED WHITE RATS. (Body-weight in grams.)

SERIES I.							
CONTROL.				EXPERIMENTED.			
No. of animals and sex.	Initial.	Final.	Difference in per cent.	Difference in per cent.	Final.	Initial.	No. of animals and sex.
(1) 4 F.	82.1	115.1	+40	-26	67.0	91.8	5 F.
(2) 2 F.	80.0	122.4	+53	-26	60.7	82.9	3 F.
(3) 3 M.	99.2	159.3	+60	-34	67.0	101.3	3 M.
(4) 2 M.	52.3	120.0	+129	-28	32.2	44.8	4 M.
(5) 3 F.	30.2	55.8	+46	-36	25.5	40.2	2 F.
(6) 2 M.	36.0	61.6	+70	-33	27.5	41.0	2 M.
SERIES II.							
(1) 1 F.	59.8	-24	50.6	66.7	1 F.
(2) 4 M.	73.2	-28	51.8	72.1	4 M.
(3) 2 F.	89.0	-22	62.8	80.1	2 F.
(4) 2 M.	98.1	-32	65.8	96.6	3 M.
(5) 2 F.	88.9	-25	67.4	90.1	2 F.
(6) 2 F.	78.8	-28	60.1	84.0	2 F.
AVERAGE LOSS ACCORDING TO SEX. (Both series.)							
..	-26.7	17 F.
..	-31.7	16 M.

must be kept in mind, for previous work has mainly dealt with the complete starvation of mature animals. In the present case, the par-

tially starved white rats lost only from 22 to 36 per cent of their body-weight in the course of twenty-one days. If Chossat's hypothesis that starved animals die after they have lost two-fifths of their original body-weight is correct, the white rats in the present instance might have lived some five or six weeks longer. The daily loss in body-weight is not regular throughout the period of the experiment, but varies markedly according to the changes in temperature and

TABLE II.
SHOWING BRAIN-WEIGHT IN BOTH CONTROL AND EXPERIMENTED WHITE RATS.
(Body and brain-weight in grams.)

SERIES I.							
CONTROL.				EXPERIMENTED.			
No. of animals and sex.	Initial.	Final.	Brain-weight.	Brain-weight.	• Final.	Initial.	No. of animals and sex.
(1) 4 F.	82.1	115.1	1.678	1.595	67.0	91.8	5 F.
(2) 2 F.	80.0	122.4	1.783	1.547	60.7	82.9	3 F.
(3) 3 M.	99.2	159.3	1.781	1.562	67.0	101.3	3 M.
(4) 2 M.	52.3	120.0	1.645	1.400	32.2	44.8	4 M.
(5) 3 F.	30.2	55.8	1.407	1.302	25.5	40.2	2 F.
(6) 2 M.	36.0	61.6	1.472	1.320	27.5	41.0	2 M.
AVERAGE. (Difference in per cent.)							
9 F. + 7 M.	1.628 (+ 11.0 %)	1.454	10 F. + 9 M.

other surrounding conditions which may act unfavorably. Generally speaking, the greater loss in weight occurs during the first few days, and after that, unless the surrounding conditions are rendered suddenly unfavorable, the daily loss is quite regular. Unlike those undergoing the complete starvation, the animals suffer from severe constipation, and as a consequence the loss in body-weight becomes irregular. Table I shows the initial and final body-weights of both the control and experimented groups.

As Table I shows, both the gains in the growing rats and the losses of those starved are quite irregular, ranging from 40-129 to 22-36 per cent, respectively. Generally speaking, the male rats lose during starvation proportionally more than females, as the figures given above show (— 27 per cent in female and — 32 in male).

On brain-weight. — It has been shown in the preceding section that the white rats fed on fat and starch have lost during three weeks from 27 per cent (average for females) to 32 per cent (average for males) in body-weight. We now wish to determine whether the brain has taken part in this loss of weight. To answer this question, the rats belonging to both control and experimented groups were killed, and the brain was removed and weighed. Table II shows brain-weight in both groups.

In analysing Table II, Series I and Series II must be considered separately, since the arrangement of the experiment was different in the two series.

From Series I, it is seen that the control rats have a much heavier brain. It appears that on the average the difference in brain-weight between the control and experimented is 0.174 gram, or 11 per cent in favor of the control groups. In other words, during three weeks the control groups have gained 11 per cent in brain-weight over the experimented groups. This figure is, however, the algebraic sum of the gain in the control groups due to normal growth, plus the loss due to starvation in the experimented groups. The gain in brain-weight due to growth can be determined, of course, by comparing in the control groups the brain-weight at the beginning of experiment with that found at the end, and similarly the loss can be determined during the same period for the experimented groups. As a given animal can be killed only once, it is necessary to estimate its brain-weight at the other end of the period by an appeal to a second series of data. This is possible by the use of an extensive series of records on file in this laboratory, which give the brain-weight in white rats of different body-weights and at different ages. From these data the brain-weights corresponding to the body-weights of the rats in both the control and experimented group were calculated. The determinations thus made are shown in the following Table III. In the column headed "Initial brain-weight calculated" are found the values calculated from the laboratory records.

TABLE III
SHOWING BRAIN-WEIGHT, CALCULATED AND OBSERVED, BELONGING TO THE CONTROL AND EXPERIMENTED WHITE RATS.

CONTROL.						EXPERIMENTED.					
No. of animals and sex.	Body-weight (initial).	Body-weight (final).	Initial brain-weight calculated.	Final brain-weight observed.	Gain (dif. ference in per cent).	Loss (difference in per cent).	Final brain-weight observed.	Initial brain-weight calculated.	Body-weight (final).	Body-weight (initial).	No. of animals and sex.
(1) 4 F.	82.1	115.1	1.565	1.678	+ 7.2	-3.3	1.595	1.650	67.0	91.8	5 F.
(2) 2 F.	80.0	122.4	1.545	1.783	+15.4	-1.1	1.547	1.575	60.7	82.9	3 F.
(3) 3 M.	99.2	159.3	1.640	1.781	+ 8.5	-7.0	1.562	1.680	67.0	101.3	3 M.
(4) 2 M.	52.3	120.0	1.435	1.645	+14.6	-3.1	1.400	1.445	32.2	44.8	4 M.
(5) 3 F.	30.2	55.8	1.250	1.407	+12.5	-5.3	1.302	1.375	25.5	40.2	2 F.
(6) 2 M.	36.0	61.6	1.375	1.472	+ 7.1	-7.6	1.320	1.430	27.5	41.0	2 M.
AVERAGE ACCORDING TO AGE.											
(1) (2) { 6 F. } older	81.1	118.7	1.555	1.730	+11.2	-2.5	1.571	1.613	63.9	87.4	8 F. } 3 M. }
(3) { 3 M. } older	99.2	159.3	1.640	1.781	+ 8.5	-7.0	1.562	1.680	67.0	101.3	2 F. } 6 M. }
(5) { 3 F. } younger	30.2	55.8	1.250	1.407	+12.5	-5.3	1.302	1.375	25.5	40.2	
(4) (6) { 4 M. } younger	44.2	90.8	1.405	1.559	+10.8	-5.3	1.437	1.360	29.8	42.9	

From the above, it is seen that an actual loss in brain-weight occurs in the experimented rats. On the average this is 4.8 per cent in the case of the older rats, and 5.3 per cent in the case of the younger. In the control groups the gain in the older is 9.8 per cent, and in the younger 11.6 per cent.

To check the above results, a second series of rats was tested in the following way. Animals belonging to the same litter were divided into two groups, as in Series I. The individuals used for control and experiment were selected in such a way that the average body-weights were approximately the same in both groups. In this series, the control groups were killed at the beginning of the experiment, and the experimented groups alone were kept alive for three weeks. Thus the brain-weights of the controls at the beginning of the experiment were compared with those of the starved rats at the end. The records show that during three weeks, an undernourished rat has actually lost in brain-weight, thus corroborating the conclusion drawn from Series I. It must be remembered that the brain-weight of the white rat is a function of the body-size (usually measured by its weight), and is dependent on the age only so far as that affects the size of the animal (Donaldson and Watson). Therefore, if one selects for control and experiment, animals having the same body-weight, then the brain-weights in two series of animals should correspond. The results obtained from the second series are contained in the table on the following page.

As Table IV shows, the results obtained from Series II confirm those based on Series I. The average initial body-weights in both control and experimented groups are approximately the same. Therefore the initial brain-weights in the two groups were approximately the same, but in the experimented groups the final weight found after three weeks' starvation was slightly below that of the control, indicating an actual loss in brain-weight. The loss in percentage is 5.8 per cent in the male, and 2.8 per cent in the female. The average from these two is 4.3 per cent, which is to be compared with 4.75 per cent as shown by the older set in Series I. This percentage loss in the brain-weights of the experimented rats is much higher than that obtained by the previous investigators. Before making a comparison with our own results, it will be necessary to state the conditions under which the experiments by other observers were made.

According to Voit,¹ a cat which had died as the result of a

¹ C. v. VOIT: Hermann's Handbuch der Physiologie, 1889, vi, pp. 95-103.

complete withdrawal of food and water (time not given) showed a brain-weight slightly lighter than that of the control animal. The difference in weight being about 3 per cent in favor of the control. This observation by Voit is the only record of the effect of starvation on the brain-weight of a mammal which we have been able to find. It is to be noted, moreover, that it applies to an animal subjected to

TABLE IV.

SHOWING BRAIN-WEIGHT IN BOTH CONTROL AND EXPERIMENTED WHITE RATS.

(Controls killed at the beginning of the experiment. Body and brain weight in grams.)

SERIES II.							
CONTROL.				EXPERIMENTED.			
No. of animal and sex.	Body-weight (initial).	Body-weight (final).	Brain-weight.	Brain-weight.	Body-weight (final).	Body-weight (initial).	No. of animal and sex.
(1) 1 F.	57.8	..	1.380	1.363	50.6	66.7	1 F.
(2) 4 M.	73.2	..	1.488	1.420	51.8	72.1	4 M.
(3) 2 F.	89.0	..	1.586	1.487	62.8	80.1	2 F.
(4) 2 M.	98.1	..	1.616	1.502	65.8	96.6	3 M.
(5) 2 F.	88.9	..	1.635	1.569	67.4	90.1	2 F.
(6) 2 F.	78.8	..	1.538	1.545	60.1	84.0	2 F.
AVERAGE ACCORDING TO SEX. (Difference in per cent.)							
6 M.	85.7	..	1.552 (-5.8%)	1.461	58.8	84.4	7 M.
7 F.	79.1	..	1.535 (-2.8%)	1.491	60.2	80.2	7 F.

complete starvation, and hence living during the period of experiment probably less than thirteen or fourteen days. Chossat,¹ who examined the brain-weight in the two pigeons dying of starvation, found the weight of the brain only 1.9 per cent less than that of the controls. The pigeons lived without food or water for 13 days. The

¹ C. CHOSSAT : Mémoires présentés par divers savants à l'académie royale des sciences de l'institut de France, 1843, viii, p. 438.

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TABLE V.
SHOWING THE PERCENTAGE OF WATER IN THE BRAIN.

SERIES I.							
CONTROL.				EXPERIMENTED.			
No. of animals and sex.	Body-weight (final)	Brain-weight (final).	Per cent of water.	Per cent of water.	Brain-weight (final).	Body-weight (final).	No. of animals and sex.
(1) 4 F.	115.1	1.678	78.93	78.54	1.595	67.0	5 F.
(2) 2 F.	122.4	1.783	78.96	78.82	1.547	60.7	3 F.
(3) 3 M.	159.3	1.781	78.73	78.65	1.562	67.0	3 M.
(4) 2 M.	120.0	1.645	79.46	79.15	1.400	32.2	4 M.
(5) 3 F.	55.8	1.407	79.33	79.18	1.302	25.5	2 F.
(6) 2 M.	61.6	1.472	79.28	79.13	1.320	27.5	2 M.
AVERAGE ACCORDING TO AGE.							
(1)+(2)+(3) older.	132.3	1.747	78.87 (-0.20)	78.67	1.568	64.9	
(4)+(5)+(6) younger.	79.1	1.508	79.36 (-0.21)	79.15	1.341	28.4	
SERIES II.							
(1) 1 F.	59.8	1.380	79.22	78.77	1.363	50.6	1 F.
(2) 4 M.	73.2	1.488	79.42	79.18	1.420	51.8	4 M.
(3) 2 F.	89.0	1.586	78.78	78.57	1.487	62.8	2 F.
(4) 2 M.	98.1	1.616	78.47	78.37	1.502	65.8	3 M.
(5) 2 F.	88.9	1.635	78.59	78.48	1.569	67.4	2 F.
(6) 2 F.	78.8	1.538	79.59	78.90	1.545	60.1	2 F.
AVERAGE ACCORDING TO AGE.							
Older.	79.01 (-0.3)	78.71			

higher percentage loss in brain-weight in the animals studied by us may be explained as dependent on three special conditions. (1) The animals used were still actively growing;¹ (2) During the experiment, the animals suffered from severe constipation. Such a disturbance might produce substances injurious to the brain, and thus assist in arresting its growth, or actually cause it to lose weight. (3) The length of time during which the animals lived was greater than in the cases of complete starvation.

Percentage of water.—Since the brain, like other organs, is composed of water and solids, the loss in weight must be due to either a diminution of water or solids or both. A determination of water in the brain is, therefore, of interest in connection with the loss of brain-weight. For this determination, the brain, after its fresh weight had been taken, was dried for one week at a temperature of 90° C. and the percentage of water thus found. As a result of this treatment, the results tabulated on the preceding page were obtained.

From this table, it will be seen that in Series I the percentage of water in the brain, belonging to the control groups, is slightly higher than that in the experimented groups. On the average the percentage of water in the experimented groups is 0.2 less for the older animals, and 0.21 less for the younger animals. We see then in groups of the same age, and in which we should expect the same percentage of water in the brain, that the experimented groups give a slightly lower figure, which, however, appears in every case. There is, therefore, an actual diminution of water in the brain, in the experimented groups.

In the case of Series II, we found, on the average, that the experimented rats have a percentage of water in the brain which is 0.3 less than that of the controls, with which they were assumed to correspond, but which were killed at the beginning of the experiment. Thus the loss of water in the experimented groups of Series II is 0.1 case, however, we must remember the influence of age on the diminution of water as stated in the foot-note on the previous page. Since greater than that of the corresponding groups of Series I. In this the experimented groups in Series II, lived three weeks longer than the controls, the loss of 0.3 is the sum of the diminution due to the starvation and that due to the advance in age. Although we do not

¹ The percentage of water in the nervous system is a function of age, and is independent of either the absolute size of the brain or weight of the body. The percentage diminishes with age.

know exactly how much of the loss is due to the age, yet the results from Series I would suggest that the greater part was due to starvation.

A very slight percentage loss of water in the brain of starved animals, was also found by Lukjanow.¹ Lukjanow selected twenty pigeons for the control, and another twenty having the same body-weight as the former, for experiment. He killed the control pigeons at the beginning of the experiment, and determined the weight of the brain, and the percentage of water and of solids in it. The experimented pigeons were kept until they died. The percentage of water in them was then determined. He obtained on the average 79.94 per cent (male), and 80.37 per cent (female) in the control group, and 79.73 per cent (male) and 79.82 per cent (female) in the experimented group. Thus the difference in percentage of water between the two groups is 0.21 for the males, and 0.55 for the females, or 0.48 on the average for both sexes.

The loss of water in the starved pigeons is slightly higher than that obtained from our own experiments on the white rat. The difference may be due to the fact that the method (*i. e.* complete starvation) and the animals employed by Lukjanow were different from those used by us. It is interesting, however, to note that the percentage of water in the brain is slightly affected even in the pigeon, which, as the representative of a class below the mammals, might be expected to be less stable.

From unpublished observations in this laboratory it has been concluded that during normal growth the percentage of water in the brain of the rat is a function of its age, and is not directly modified by the brain or body weight (Donaldson). This conclusion may be extended by the present experiment to cover cases of growth under abnormal conditions, since the percentage of water, in both control and experimented groups, is nearly the same, differing only by 0.2. As an example, we refer to the group records in Table V. For instance, in Table V, Series I, Group 4, the final body-weight of the control rats is nearly four times that of the corresponding experimented rats (120 to 32.2 gms.) yet the percentage of water in the two lots of brains is 79.46, and 79.15 per cent, respectively. In all other cases, of Series I, the control groups weigh more than twice as much as the experimented, and yet, as we have seen, the percentage of water in the brain differs but very slightly.

¹ S. M. LUKJANOW: *Zeitschrift für physiologische Chemie*, 1889, xiii, pp. 339-351.

On extracts.—The diminution of the percentage of water in the brain as the result of inadequate nutrition is due to a diminution of lymph or to a breaking down of the solid substance or to both. An increase of water extractives from the body of starved animals, indicates an extensive destruction of solids.¹ Further investigation of the solids obtained from the brain is, therefore, important in order to determine the nature of the destructive effects resulting from the present experiment.

Broadly speaking, the dried solids from the nervous system are composed of the two classes of substances: namely, those soluble in ether and alcohol, and those insoluble in these reagents. The soluble substances or extractives are mainly represented by the so-called "myelin," which is contained in the medullary sheath, and the insoluble substances are represented by protein bodies. Variation in the percentage value of the extractives indicates which substances have been altered. In the present case, the dried solids were extracted with boiling 95 per cent alcohol and ether. The process of extraction was as follows: (1) Boiling alcohol (95 per cent) for three days—the alcohol was changed once daily. (2) Cold ether—for 48 hours—changed twice. (3) Boiling alcohol (95) per cent, for 24 hours. After the six days treatment, as indicated above, the residue was collected, dried, and weighed. From this test the following results were obtained:

TABLE VI.

SHOWING AMOUNT OF EXTRACTIVES IN PER CENT.

(In Series I, the controls were killed at the end of the experiment, and are the same age as the experimented. In Series II, the controls were killed at the beginning of the experiment, and are twenty-one days younger than the experimented.)

SERIES I.					SERIES II.			
	OLDER.		YOUNGER.		OLDER.		YOUNGER.	
	Extract in per cent.	Differ- ence.	Differ- ence.	Extract in per cent.	Extract in per cent	Differ- ence.	Differ- ence.	Extract in per cent
Control . .	47.1	45.8	48.06	45.8
Experimented	47.6	+ 0.5	+ 0.9	46.7	49.45	+ 1.39	+ 0.9	46.7

¹ B. SLOWTZOFF: Beiträge zur chemischen Physiologie und Pathologie, 1904, iv, pp. 23-39.

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From the above table, it is seen that in the case of Series I, the percentage of extractives in the experimented groups is slightly higher than the control. On the average, the experimented rats give 0.7 per cent more than control. This difference is relative merely, since the control groups show the larger brains, and hence give the greater absolute amount of extract. The percentage value of the extractives increases with the age of the animal, and the real increase in percentage can be determined only by comparing the percentage of extractives obtained at the beginning with that found at the end of the experiment. For this purpose the solids obtained from Series II were used and the results are given on the left side of the same table. From this it appears that the difference in the amount of extractives in per cent, between the control and experimented groups, is on the average 1.14, in favor of the experimented groups. This difference appears in rats which were starved and were twenty-one days older than the control groups, hence, we conclude that the increase in percentage due to the starvation is in Series II similar to that in Series I, *i. e.*, approximately 0.7; while the difference between 0.7 and 1.14, or 0.44, is to be credited to the greater age of the starved rats in Series II. As the absolute weight of the brains in the starved groups has diminished, and as the relative amount of the extractives has been increased, we infer that the protein substances have been most affected, as the result of partial starvation. This being the case, we might expect to find changes in the cell bodies. Examination of our own material has not revealed any marked alteration in the Nissl substance of the cells of the brain. The point is, however, open to further investigation. In animals allowed to starve to death, Tauszk, in 1896,¹ and Jacobsohn, in 1897,² and Marchand and Vurpas³ have reported changes in the cell-bodies. The search for changes in the medullary substances has given negative results in the hands of Von Merzbacher,⁴ and of Donaldson and Hoke (unpublished observation). However, to make the comparison complete, we should also know the percentage value of the ether and alcohol extracts in the case of the animal dying from starvation, but for this we shall be obliged to wait until experiments to test that point have been completed.

¹ TAUSZK: *Neurologisches Centralblatt*, 1894, p. 820.

² JACOBSON: *Neurologisches Centralblatt*, 1897, p. 946.

³ L. MARCHAND and O. VURPAS: *Comptes rendus de la Société de biologie*, 1900, p. 296.

⁴ V. MERZBACHER: *Archiv für die gesammte Physiologie*, 1903, C, p. 568.

ON THE REDUCING ACTION OF THE ANIMAL ORGANISM UNDER THE INFLUENCE OF COLD.

By C. A. HERTER.

A PERUSAL of Ehrlich's extremely suggestive but neglected work, entitled "Das Sauerstoff-Bedürfniss des Organismus,"¹ led me to undertake the application of intravital staining methods to the study of some physiological and pharmacological problems.² In the present note, I desire to speak briefly of the influence of cold upon the phenomena of intravital staining with methylene blue and brilliant cresyl blue. The experiments already made indicate that the behavior of certain structures in animals subjected to cold, differs in important respects from the behavior of similar structures in animals which maintain a normal temperature. The gray substance of the central nervous system and the muscular structures are the parts which show the greatest alteration in function, especially in respect to the power of reduction, which is much diminished by cold.

When a living rabbit has been infused with a solution of methylene blue, the appearances in the organs during life and after death are influenced by a number of conditions, including the weight of the animal, the quantity of blue infused, the rapidity of the infusion, and finally the interval which elapses between the close of the infusion and the observation of the tissues of the living or dead animal. Of the internal factors which influence the appearances, the varying reducing power of the different kind of cells is the most important. The lungs, liver, suprarenals, and gray matter of the central nervous system energetically reduce methylene blue to its colorless leuco-base during life. The muscles, spleen, kidneys, and connective tissues reduce less activity. In estimating the reducing power of different sorts of cells, one cannot be guided solely by the color of the organs,

¹ Published in 1885.

² The chief results of work done during this year in the pharmacological laboratory of Prof. HANS MEYER, at the University of Marburg, will shortly be published in the *Zeitschrift für physiologische Chemie*.

for absence of color in an organ may mean that it has removed little or no dye from the blood. This view receives confirmation if, after its removal, the colorless organ does not undergo bluing on contact with air, or, better still, on the application of hydrogen peroxide or potassium persulphate. On the other hand, one may be led to underestimate the reducing activity of an organ, if one considers only its blue color during life. For example, the blueness of the kidney during a methylene blue infusion depends partly on the circumstance that a very large quantity of the dye passes into its cells on the way to elimination. Although interest attaches to a consideration of typical color-pictures of the organs during variously conditioned intravital infusions, a full description of such normal appearances is unnecessary for the purpose of the present note, for the reason that each experiment on the influence of cold (excepting one ¹) has been made with the aid of a control experiment upon a normal animal.

The control experiments were made simultaneously with the experiments on the cooled animals, and thus afforded a satisfactory means of checking the results, as they avoided the necessity for carrying the color shades in the memory. Although the intravenous infusions were made under different conditions, as regards the rate, concentration, and volume of the infusion, the temperature, the weight of the animal, and the duration of life after the close of the infusion, the control experiments were, in all instances, carried on under conditions comparable with those present in the corresponding experiments upon cooled rabbits. The cooling was accomplished by means of cold wet cloths, with or without the use of ice.

The intravenous infusions, which were made into the jugular vein, were not begun until the animal had been considerably cooled, and the cooling was generally maintained until the time of autopsy. The methylene blue (Grubler's) was dissolved in 0.85 per cent salt solution, and was infused at the room-temperature or at a temperature a little above this.

Most of the important results respecting the appearance of the organs have been embodied in the accompanying table.

There are three features of the tabulated experiments which deserve especial attention; one of these relates to the appearance of the muscles. If the pectoral muscles of a cooled animal be inspected after the infusion of a small quantity of methylene blue solution (say

¹ Experiment 1. The notes on the control experiment in this instance were mislaid, but it was the contrast observed which led to the series reported here.

15-20 c.c. of a 0.25 per cent solution), they are found to be blue in color, the tint deepening with the volume and rapidity of the infusion. The muscles of the control animal are likewise blued, but the coloration is less (as a rule) than in the cold animal. After the close of the infusion, the blue present in the muscles of the normal animal is gradually reduced to leuco-methylene blue, whereas the reduction is distinctly slower in the case of the cooled rabbit. In the experiments recorded in the table, the animals were not killed until a decided difference existed in the tints of the two sets of muscles. After death, reduction proceeds energetically in the muscles, but the reduction is more rapid in the uncooled rabbit.

On exposure to the air or after treatment with oxidizing agents, there is a return of color in the muscles of the normal animals, which indicates that the differences in color just mentioned are due chiefly, if not wholly, to differences in the power of reduction, and not largely to the transportation of the dye. What has been said of the pectoral muscles holds true of most other skeletal muscles, and applies to the heart and diaphragm. The differences in reducing powers are often most striking in the case of the occipital muscles. Occasionally (as in Experiment 7) the differences in the reducing activity of the pectoral muscles is slight. In this case, however, the heart and occipital muscles of the cooled animals were greatly retarded in their reducing action.

Another constant feature is a marked lessening of the reducing activity of the gray substance of the brain, cerebellum, and other central nervous organs. In some instances (as in Experiments 2 and 7), the contrast between the color of the brain of the cooled animal and that of the control has been in a high degree striking, and in all the experiments, the difference in color has been distinct. It is easy to show that the color contrasts observed in the case of the central nervous system are due to difference in reducing activity, and not to differences in the quantity of blue arrested in the gray matter, for the colorless normal brains blue considerably on the application of an oxidizing agent to the freshly exposed surface. The color developed in this way is often a pure blue, whereas the color of the unreduced dye in the brain of the cooled rabbit has usually a violet shade. The reason for this difference is obscure.

A further point of some interest is the observation that when the brain of a cooled animal is very deeply colored, the medullary portion of the suprarenal body is likely to grow blue or green-blue on

exposure to the air. I have noted a similar parallelism in the appearance of the medulla of the suprarenal and gray substance of the central nervous system in a variety of toxic states. In the case of the cooling experiments, it is not clear that the coloration of the medullas, as contrasted with the coloration of the medullas of the normal animals, is due to differences in reducing power, for experiments with oxidizing agents make it likely that the medullary part of the normal suprarenal takes up less dye from the blood than does the medulla of the cooled animal.

If toward the end of an infusion of methylene blue the liver of a cooled animal be compared with the liver of the control rabbit, it will usually be found that the latter is purple-red in color, while the former is red. A similar difference in color is noticeable after death, and can easily be shown to depend on the presence of unequal quantities of methylene blue in the livers. In Experiments 5, 6, 7, and 8, the bile was examined with the following results. The quantity of bile found in the gall-bladder was about the same in the cooled and in the normal rabbit, but on dilution with water it was evident that the normal bile contained more free methylene blue than its fellow. On standing in the air, this difference was usually heightened, owing to the presence of leuco-methylene blue. A still more striking difference between the biliary secretions was brought out by boiling with dilute hydrochloric or acetic acid, which frees the leuco-methylene blue which has been paired in the liver with a substance of which the nature is at present unknown¹ (possibly glycuronic acid). After boiling the diluted bile of the control rabbits with hydrochloric acid, the addition of a few drops of hydrogen peroxide was followed by the appearance of a considerable amount of new methylene blue; the same process applied to the diluted bile of the cooled rabbits showed that little or no paired leuco-methylene blue was present. In other words, the bile of a rabbit which has been cooled excretes less free methylene blue, less leuco-methylene blue, and less paired leuco-methylene blue. On the other hand, the examinations of extracts of the livers showed that the cooled livers are apt to show somewhat more of the paired leuco-methylene blue than do extracts from the control livers, but these results were somewhat variable.

¹ The existence of paired leuco-methylene blue in the liver and kidneys was for the first time observed in the Pharmacological Institute of the University of Marburg, in the laboratory of Prof. HANS MEYER, in May, 1904.

TABLE RECORDING APPEARANCES OF ORGANS AFTER

No. of exp.		Wt. of rabbit.	Temperature (rectal). C.	Period of infusion.	Solution infused.	Volume of solution.	Interval between infusion and autopsy.	Muscles.	Liver.
1	+	grams 1380	30°-32°	min. 25	per cent 0.33 M.B.	c.c. 50.0	min. 70	Pectorals, diaphragm, and heart deep blue.	Purple.
2	+	1270	30°-32°	30	0.33 M.B.	50.0	130	Skeletal muscles deep blue; heart deep blue.	Bluish purple before exposure.
	C	1360	39°-40°	25	0.33 M.B.	50.0	130	Skeletal muscles uncolored or faintly colored; heart unstained.	Red, gives purple on exposure.
3	+	1380	31°-32°	7	1.00 B.C.B.	7.0	28	Skeletal muscles deep blue-green.	Dark red.
	C	1320	Normal 38°-39°	7	1.00 B.C.B.	7.0	2	Skeletal muscles colorless or faintly colored.	Deep blue.
4	+	1300	30°	7	0.5 B.C.B.	7.0	15	Skeletal muscles well blue.
	C	1290	38°-39°	6	0.5 B.C.B.	6.0	2	Skeletal muscles nearly colorless.
5	+	About 2000	30°	30	0.25 M.B.	21.5	20	Most skeletal muscles green-blue.	Purple.
	C	About 2000	36°-38.5°	30	0.25 M.B.	21.0	20	Most skeletal muscles colorless.	Normal red color.
6	+	1590	32°-33°	21	0.25 M.B.	21.0	15	Most skeletal muscles decided blue.	More purple than control (difference not great).
	C	1490	37.5°-38°	20	0.25 M.B.	20.0	15	Most skeletal muscles unstained or faintly tinged. Heart markedly blue; occipital muscles very deeply stained; other muscles considerably stained.	Normal red color.
7	+	1726	24°-30°-	25	0.33 M.B.	38.0	100		Purple.

+, Experiments on cooled rabbits.
C, Experiments on normal rabbits.

INTRAVENOUS INFUSIONS OF METHYLENE BLUE.

Pancreas.	Kidneys.	Suprarenals.	Brain.
Deep turquoise blue.	Cortex and medulla extremely deep blue.	Deep blue before exposure to air.
Deep blue.	Extremely blue.	Gray substance, deep blue before exposure. Does not deepen.
Deep blue.	Cortex, slightly blue; deepens much on exposure.	Very slight bluing of gray matter, deepening on exposure.
Deep blue.	Cortex purple.	Medulla blue on exposure.	Slightly colored, but on exposure grows deep purple-blue.
Blue on exposure to air.	Colorless.	Blue deeply on exposure.	Colorless; on exposure colors moderately.
....	Gray matter, blue before exposure to air.
....	Gray matter slightly blued; blues on exposure to air.
....	Nearly colorless, but blues slightly on exposure.	Cortex colorless.	Gray matter moderately blue throughout before exposure.
....	Blue, deepening on exposure.	Medulla blues deeply on exposure.	Colorless gray matter; blues somewhat on exposure.
Deep turquoise blue.	Cortex deep blue. Papillæ pale.	Cortex and medulla quite colorless.	Pale blue, on opening skull.
Almost unstained.	Cortex moderately blue. Papillæ blue deeply on exposure.	Colorless.	Colorless; on exposure to air gray matter blues faintly.
Deep blue; somewhat deeper than control.	Cortex deep blue. Medulla less blue.	Cortex colorless. Medulla instantly blues deeply on exposure.	Gray matter, everywhere deep purplish blue before exposure to air.

M.B., Methylene blue.

B.C.B. Brilliant cresyl blue.

TABLE RECORDING APPEARANCES OF ORGANS AFTER

No. of exp.		Wt. of rabbit.	Temperature (rectal), C.	Period of infusion.	Solution infused.	Volume of solution.	Interval between infusion and autopsy.	Muscles.	Liver.
		grams		min.	per cent	c.c.	min.		
	C	1786	37.5°—	26	0.33 M.B.	40.0	100	Heart slightly blue; occipital muscles nearly colorless; other muscles considerably or moderately stained.	Red.
8	+	1610	30°–32°	28	0.25 M.B.	31.0	10	Pectorals and diaphragm deep blue.	Purple.
	C	1580	38°	30	0.25 M.B.	30.0	10	Pectorals and diaphragm faint green-blue.	Red.
	+	1295	30°–32°—	24	0.25 M.B.	24.0	10	Pectoral and diaphragm moderately blue.	Purple.
9	+	1460	23°–30°	60	0.33 M.B.	30.0	5	Pectoral and otherskeletal muscles markedly blue; heart very blue.	Purple.
	C	1550	38°	60	0.33 M.B.	31.7	5	Pectoral and otherskeletal muscles slightly blue; heart slightly blue.	Red.
10	+	2280	27°–30°	62	0.33 M.B.	41.0	5	Pectoral and otherskeletal muscles considerably blue; heart deeply blue.	Deep purple; olive-green on exposure to air.
	C	2733	38.5°—	62	0.33 M.B.	50.0	5	Pectoral and otherskeletal muscles moderately blue; heart moderately blue.	Red.

+, Experiments on cooled rabbits.
C, Experiments on normal rabbits.

INTRAVENOUS INFUSIONS OF METHYLENE BLUE—*continued.*

Pancreas.	Kidneys.	Suprarenals.	Brain.
Deep blue.	Cortex deep blue. Medulla less blue.	Colorless.	Gray matter, everywhere quite colorless; blues on exposure to air.
Turquoise blue.	Cortex deep blue. Medulla slightly stained. Papillæ unstained.	Colorless.	Gray matter markedly blue before exposure to air.
Very pale blue.	Cortex moderately blue. Medulla moderately blue. Papillæ deep blue on exposure.	Colorless.	Gray matter, very pale green; blues slightly on exposure.
Very pale blue.	Cortex marked blue. Medulla slightly stained. Papillæ unstained.	Colorless.	Gray matter slightly blue; color deepens on exposure.
Deep turquoise blue; slightly deeper than control.	Cortex deep blue. Medulla and papillæ only slightly stained.	Cortex uncolored. Medulla green; blues on exposure.	Gray substance markedly blue before exposure to air.
Deep turquoise blue.	Cortex moderately blue. Medulla and papillæ markedly blue.	Cortex and medulla colorless.	Colorless; blues considerably on exposure to air.
Deep turquoise blue.	Cortex deep blue. Medulla pale.	Cortex and medulla colorless. Medulla moderately green on exposure.	Markedly blue before exposure; deepens somewhat on oxidation.
Pale blue.	Cortex and medulla deep blue.	Cortex and medulla colorless. Medulla slightly green on exposure.	Slightly blue before exposure; deepens on oxidation.
M.B., Methylene blue. B.C.B. Brilliant cresyl blue.			

The stomach and small intestines of the cooled animals are generally more blue than the same parts in the control animals. The pancreas may be much more blue in the cooled animal than in the control (Experiments 6 and 8), but sometimes the differences are slight (Experiment 9). The kidneys vary considerably in appearance, and these variations appear to be connected with the state of renal secretion. As a rule the normal kidneys have secreted more methylene blue, more leuco-methylene blue, and more paired leuco-methylene blue than the cooled animals. If the autopsy be made soon after the close of the infusion, the cortex of the kidney will be found to be deeply stained, and the medullary parts, at first pale blue, will be seen to blue deeply on exposure to the air. On the other hand, the kidneys of cooled rabbits are often seen to be even more deeply stained than the normal cortices, while the medullary parts are pale, and do not blue on exposure to the air, or blue only slightly. In a few instances (9 and 10), the lungs of the cooled animals have been observed to be slightly blue during life as well as after death.

By means of an intravital staining method such as I have employed in the experiments here reported, it is possible not merely to localize with precision the parts in which function has been modified, but also to obtain a rough conception of the degree to which function has been modified in individual structures. In these experiments, the temperature of the cooled animals has often been lowered as much as 10° C. below that of the normal control animal. The effect of temperature on the velocity of chemical reactions has been studied between 0° C. and 200° C., in many instances, and it is known that the reaction velocity of simple chemical reactions is doubled or tripled through every increase of 10° — C. in temperature. As the processes of oxidation and reduction in the animal organism are intimately associated with the activity of catalytic enzymes, the velocity of such processes has to be estimated in the light of the facts relating to the velocity of catalytic reactions, rather than on the basis of observation pertaining to simple chemical reactions. Careful observations relating to the influence of temperature on the rapidity of catalytic processes in living organisms are still scanty. The studies of Clausen upon the excretion of carbon dioxide by bean-germs, wheat-germs, etc., and the work of Hertwig on the development of frog's eggs indicate that within certain limits of temperature (0° — 25° C. in the former case, 6° — 24° C. in the latter) the increase in reaction-velocity is as great as in the case of simple chemical

reactions. Although we cannot transfer these results to warm-blooded animals, they are of interest in relation to the present experiments, as evidence that temperature affects the rapidity of enzyme-induced chemical processes in a degree not necessarily very different from ordinary chemical reactions. The slowing of the heart, the secretion of bile and of urine (even quite aside from the color picture), all point to a pronounced lessening, both of oxidations and of reductions, under the influence of a decline of $10^{\circ} - C.$ in body-temperatures. Intravital staining experiments, conducted simultaneously with the determination of the respiratory metabolism, should prove highly instructive in this connection.

By means of the intravital method of staining as employed in the experiments which form the basis of this note, it is a simple thing to make a striking class demonstration of the influence of cold on processes of reduction, a demonstration which it would be difficult to secure by any other method. The most satisfactory results are to be obtained by those infusions in which the color is rather slowly introduced, — say 1 c.c. of a 0.33 per cent methylene blue solution in two minutes. Under these circumstances, the muscles of the control rabbit not only blue more intensely than those of the cooled animal, but the bluing occurs considerably earlier in the course of the infusion. Where an infusion has been slowly made the difference between the cooled and the normal animal are considerable at the end of the infusion; further differentiation occurs in the course of the next half hour, but good results (*i. e.*, sharp differences in color) may be obtained if the animals are killed five, ten, or twenty minutes after the close of a slow infusion. These remarks refer to infusions of 35—40 c.c. of a 0.33 per cent methylene blue solution in rabbits weighing about 1500 gms. The bluing which occurs in the course of the infusion may be conveniently watched in the exposed pectoral muscles. If these muscles are being observed, some care should be taken to prevent access of air, which tends to reoxidize the reduced blue.

In two experiments made with brilliant cresyl blue (Experiment 3 and 4) the results were similar to these noted with methylene blue, allowance being made for the easier reduction of this dye.

It is my intention to study the processes of reduction in febrile conditions by means of the methods here employed in the study of the effects of cooling.

The conclusion which it is especially desired to emphasize is the

following: A considerable fall in body-temperature is attended by a diminished reduction of methylene blue to leuco-methylene blue and this result is particularly striking and unequivocal in the case of the muscles (including the heart and diaphragm) and the gray substance of the central nervous system.

THE EFFECT OF THE INTRAVENOUS INJECTION OF FORMALDEHYDE AND CALCIUM CHLORIDE ON THE HÆMOLYTIC POWER OF SERUM.

By C. C. GUTHRIE.

[From the Hull Physiological Laboratory, University of Chicago.]

IT has been shown that the hæmolytic power of foreign serum may be destroyed or attenuated *in vitro* by the addition of small quantities of formaldehyde,¹ calcium chloride and other substances.²

It seems therefore of interest to investigate the effect of the intravenous injection of formaldehyde and calcium chloride.

I.

It is essential to determine first, whether certain factors, such as hemorrhage, asphyxia, etc., which might unavoidably be introduced into the experiments, exert any influence on the hæmolytic activity of the serum.

For this purpose, a series of eight dogs was used (Experiments 1 to 8, page 141).

The dogs were etherized, and a cannula tied into the external jugular vein, and connected with a burette which contained the solution to be injected; another cannula, tied into the common carotid artery, was used for drawing blood. After drawing a normal sample of blood, the injection was made, immediately after which another sample was drawn. Further samples were drawn at five minute intervals, until a sufficient number had been taken. The blood was received into thick tubes, from twenty to thirty cubic centimetres

¹ GUTHRIE: This journal, April, 1903, ix, p. 79, and June, 1903, ix, pp. 191-193; RUEDIGER: Journal of the American Medical Association, October, 1903, xli, 16, p. 964.

² HEKTOEN: Proceedings of the Chicago Pathological Society, May and June, 1903, v, p. 303; STEWART: Journal of medical research, June, 1902, viii, p. 268.

being drawn into each. After coagulation occurred, the clots were loosened from the sides of the tubes, which were then placed in an icebox until a sufficient quantity of serum had separated.

Washed corpuscles from the rabbit, pig, and guinea-pig were prepared in the usual way, by washing defibrinated blood repeatedly with 0.9 per cent sodium chloride solution, and then making a 5 per cent suspension of the corpuscles in 0.9 per cent sodium chloride solution. These sera, for use as complement, were obtained by clotting portions of their respective bloods.

Putrefaction was avoided by observing cleanliness in all the manipulations, and by keeping the sera and corpuscles cold until actually ready for mixing, after which they were placed in an incubator at 40° C., and removed from time to time for inspection. In most instances they were centrifugalized, then inspected, shaken thoroughly, and returned to the incubator. The entire process seldom required longer than from one to three minutes.

It was not considered necessary to estimate the amount of hæmolysis by a colormetric determination of hæmoglobin, as, from the nature of the experiments, comparative results only were to be expected and very slight differences could not legitimately be taken into account. The time of *beginning, moderate, strong, and complete* hæmolysis was readily determined by inspection before and after centrifugalization.

The results were as follows:

1. Slight variations in both the rate of hæmolytic activity, and the total hæmolytic power of serum of different animals of the same species (dogs), for washed rabbit's corpuscles, were observed. Age, sex, weight, condition (nutrition), breed, and pregnancy appeared to have but little or no influence on the hæmolytic action of the serum.
2. Hemorrhage, asphyxia, and excess of anæsthetic (ether) have little or no effect on the hæmolytic action of the serum.
3. Subjecting dog's serum to a temperature of -5° to -10° C. for seventeen hours and then thawing, appears to have little if any effect on its hæmolytic action, for rabbit's washed corpuscles.

II.

It was now possible to proceed with the injection of formaldehyde and calcium chloride.

The general technique in these experiments was the same as in the experiments on hemorrhage, etc., reported above.

EXPERIMENTS 1-8.

Date.	No. of dog.	Wt.	Sex.	Age.	Condition (nutrition).	Breed.	Time etherized.	Time blood samples <i>a</i> were drawn.	Time blood samples <i>b</i> were drawn.	Treatment of animal between time of drawing samples.
June 6	1	kilos 9.0	Female	Adult	Fat	Terrier	p.m. 3.00	p.m. 4.12	p.m.
"	2	15.0	Male	Young adult	Medium (mangy)	Black cur	3.00	5.38	5.44	Bled 600 c.c.
"	3	20.6	Male	Adult	Good	Cross between coach and bull	2.30	5.44	5.48	Bled 900 c.c.
"	4	11.3	Female	Adult	Medium	Yellow cur	2.15	4.28	5.57	Gave excess ether.
"	5	15.0	Male	Adult	Good	Collie	2.45	5.19	5.28	Bled to death.
"	6	13.6	Female	Adult	Very fat, pregnant, containing 5 or 6 well-developed embryos	Terrier	2.20	4.22	5.33	Asphyxiated.
"	7	10.9	Male	Adult	Medium	White cur	2.45	4.58	5.02	Asphyxiated.
"	8	7.9	Female	Young adult	Medium	White cur	2.30	4.38	4.41	Bled 700 c.c.

Secured but the one sample of blood from Dog 1. After drawing *b*, allowed Dog 7 to partially recover, and then gave excess of ether; and at 5.14 p. m. drew *c*. (See Experiment 9 for additional data.)

Experiment 9. June 7.—Made a 5 per cent suspension of rabbit's washed corpuscles (S). 12.04 P. M. Put 0.1 c.c. of each of the sera in Series a, in tubes correspondingly labelled, and to each added 0.5 c.c. of S. The numerals at the heads of the columns indicate the time of inspection in minutes after putting in the incubator at 40° C. The numerals under these headings express the comparative degree of hæmolysis, 1 being used to indicate in which of the tubes the action was strongest, 2 the next strongest, and so on. When two or more tubes were the same, the same numeral was given to each.

Tube No.	Time after mixing in mins. 3	Time after mixing in mins. 19	Time after mixing in mins. 82	Time after mixing in mins. 236
1a	3	5	4	4
2a	4	1	3	1
3a	2	2	1*	1
4a	1	3	4	1
5a	8	8	5	2
6a	5	6	2	1
7a	4	4	2	3
8a	5	7	4	1
* Completely hæmolysed.				

The sera in Series b and Tube c were tested at the same time and in the same manner as that in Series a, with the following results:

2 a and 2 b, same; 3 a, slightly stronger than 3 b; 4 a, very slightly stronger than 4 b; 5 a, and 5 b, same; 6 a, very slightly stronger than 6 b; 7 a and 7 b, same; 7 c, slightly weaker than either 7 a or 7 b; 8 a and 8 b, same. Only slight differences occurred between any of the tubes in pairs.

Body fluids obtained from an embryo taken from Dog 6 gave the following results:

1. Peritoneal fluid was slightly hæmolytic for S.
2. Thoracic fluid was less hæmolytic for S than the peritoneal fluid.
3. Amniotic fluid had very slight or no action on S.
4. Guinea-pig's washed corpuscles were acted upon in the same way by the fluids, excepting that hæmolysis was stronger in the cases of the peritoneal and thoracic fluids.

The injection of formaldehyde (see Experiment 10) produces a moderate though decided decrease¹ in the hæmolytic power of the serum. The decrease is most marked in the serum from blood drawn immediately after the injection is finished, the hæmolytic power of the serum gradually approaching normal after the initial inhibition, which is abrupt. The decrease is still quite distinct in serum from blood drawn fifteen minutes after the injection.

Experiment 10. May 24. — Young bitch. Weight, 8.1 kilos. A freshly prepared 1.0 per cent solution of formaldehyde in 0.9 per cent sodium chloride was used for injecting. About 11 c.c. per minute produced marked respiratory and circulatory symptoms. The injection was stopped at this point each time, and about two minutes' interval allowed to elapse before injecting again, the symptoms above-mentioned largely disappearing during the interval.

6.16'30 P. M. Drew off Tube 1.

6.18. Began injecting formaldehyde.

6.30. Finished injecting.

Amount injected, 60 c.c. Estimating the blood as $\frac{1}{8}$ of the body-weight, the proportion of formaldehyde injected to blood was 1 to 900. Allowing for dilution of the blood by the solution injected, the proportion would be 1 to 1000.

6.35. Drew Tube 2, and at five minute intervals, additional specimens 3, 4, and 5.

9.33. Made 5.0 per cent suspension of rabbit's washed corpuscles (S). Put 0.1 c.c. of each serum in correspondingly labelled tubes, added 0.5 c.c. S to each, and put at 40° C.

9.38. 1, very slightly hæmolyzed; others, no hæmolysis.

9.42. 1, moderately hæmolyzed; others, slightly hæmolyzed, 2 being least, and the amount progressively increasing to 5.

9.47. 1, very strongly hæmolyzed; others, moderately, 3 being least and the others showing a progressive increase as before to 5.

9.55. 1, almost completely hæmolyzed. Others, all moderately.

10.10. Same as before as regards order. Left at room-temperature.

May 25. 4.00 P. M. 1, completely hæmolyzed, no sediment; others, strongly hæmolyzed, but not completely, as sediment is present in all. Centrifugalized and washed with 0.9 per cent sodium chloride solution.

¹ I quoted an experiment in a previous paper (This journal, 1903, ix, p. 191) in which the injection of formaldehyde produced no diminution in the hæmolytic power of dog's serum for rabbit's corpuscles. This is probably due to the fact that only about one-half the amount of formaldehyde was injected in that experiment, namely, 1:1500 against 1:900 to 1:740 in those described in this paper.

1 has very small amount of white sediment; 2, a fair amount of red sediment; and 3, 4, and 5 have about one-half the amount of red sediment as 2.

Experiment 11. Jan. 28. — Fat young male dog, weight 15.6 kilos.

4.20 P. M. Etherized. Injected 0.5 per cent calcium chloride in 0.9 per cent sodium chloride.

When the amount injected reached 30 c.c., the respiratory movements became irregular and jerky; when 40 c.c. were injected, a marked irregularity of the heart-action was observed. The proportion of calcium chloride to blood injected was about 1:4000.

4.30 to 4.33. Injected 55 c.c. calcium chloride solution.

4.34. Drew Tube 2, and subsequently, at five minute intervals, drew Tubes 3, 4, 5, and 6.

Jan. 29. Made 5.0 per cent suspension of rabbit's washed corpuscles (S).

2.40 P. M. Put 0.1 c.c. of serum from each of the tubes in correspondingly labelled tubes, added 0.5 c.c. S to each, and put at 40° C.

2.57 P. M. 1, moderately hæmolyzed; 5, very slightly hæmolyzed; others, unchanged.

3.08¹. All hæmolyzed; 1, completely; 2, least of any; obvious progressive increase in amount of hæmolysis from 2 through 6. Centrifugalized and found a progressive decrease in amount of sediment from 2 through 6.

In five other experiments performed in the same way, some of the mixtures were incubated for eighteen hours, and showed incomplete hæmolysis in the tubes containing the serum drawn after injecting calcium chloride.

Calcium chloride was injected (see Experiment 11) in such proportions as to give a concentration of the salt in the calculated amount of blood in the animal, of 1:4700 to 1:3700, the average of five experiments being about 1:4000.

The effect is much the same as that produced by formaldehyde except that the inhibition of hæmolytic power is greater for calcium chloride in the proportions injected than for formaldehyde.

III.

Mainwaring¹ states that *in vitro* calcium chloride inhibits the hæmolytic action of immune serum by acting on the complement and not on the intermediary body or corpuscles.

¹ MAINWARING: Journal of infectious diseases, 1904, i, p. 112.

This hypothesis was now tested by the intravenous injection of formaldehyde and calcium chloride (see Experiments 12 and 13).

The two usual methods of activating corpuscles were employed: namely, treatment with hæmolytic serum at 0° C. for some hours, followed by washing with cold sodium chloride solution; and incubating washed corpuscles with serum previously heated to 56° C.

A large series of careful experiments along this line were rendered useless by the fact, previously unknown to me, that normal rabbit's corpuscles treated as above with normal dog's serum are not hæmolyzed on the addition of normal rabbit's serum, and, of course, no hæmolysis occurred in rabbit's corpuscles treated similarly with serum from dogs injected with calcium chloride.

That this failure was not due to any fault in the technique is shown by the results of control experiments done in the same way on rabbit's serum and guinea-pig's corpuscles, which gave positive results by both methods. A positive result was also obtained by both methods for guinea-pig's corpuscles and dog's serum. Pig's corpuscles and dog's serum, on the other hand, gave a negative result, *i. e.*, no hæmolysis, although normal dog's serum is strongly hæmolytic for normal pig's corpuscles.

The serum used for activating corpuscles at zero, showed, after centrifugalization, a loss in hæmolytic power for normal rabbit's corpuscles. This would indicate that some hæmolytic factor was removed or destroyed by contact with the washed corpuscles. If this was the intermediary body, in Ehrlich's sense, it would seem as if rabbit's serum did not contain a substance (complement of Ehrlich) which, acting with the intermediary body, can cause hæmolysis of rabbit's corpuscles.

Experiment 12. May 25.—Put some active dog's serum in water-bath at 56° C. for thirty minutes.

Serum A was obtained from blood drawn from a dog before injection of formaldehyde.

Serum B was obtained from blood drawn from the same dog after injection of formaldehyde.

Serum 1 was obtained from blood drawn from another dog before injection of calcium chloride.

Serum 2 was obtained from blood drawn from the same dog after injection of calcium chloride.

Made 5.0 per cent suspension of rabbit's washed corpuscles (S).

11.43 A. M. Placed 0.1 c.c. of each heated serum in correspondingly labelled tubes, added 0.5 c.c. S to each, and put at 40° C.

4.45 P. M. No hæmolysis.

2.03 P. M. Removed 0.1 c.c. of the mixture from each tube, and added 0.1 c.c. of rabbit's normal serum to each, and put at 40° C.

4.45 P. M. No hæmolysis.

Experiment 13.—10.30 P. M. Centrifugalized rabbit's washed corpuscles and to 1 c.c. of the sediment added 2 c.c. dog's normal serum at 0.0° C., and left in melting ice for eleven and one-quarter hours.

9.45 A. M. Tube shows strong agglutination, but very slight hæmolysis. Washed with cold 0.9 per cent sodium chloride solution, and made 5.0 per cent corpuscular suspension (S).

10.45. Put at 40°.

(1) 0.5 c.c. S + 0.1 c.c. rabbit's serum.

(2) 0.5 c.c. S + 0.1 c.c. 0.9 sodium chloride.

11.15. 1, no hæmolysis; 2, slight.

4.10 P. M. 1, very slight or no hæmolysis; 2, marked hæmolysis.

Numerous other experiments with dogs' sera and rabbits', guinea-pigs' and pig's corpuscles, and rabbits' sera and guinea-pig's corpuscles were performed in practically the same manner. Larger quantities of the serum in proportion to the corpuscles were added, with the same results. The length of time the foreign serum was allowed to act upon the washed corpuscles or blood, at zero C., varied from one to sixteen hours.

Otherwise I am unable to explain the cause of the negative results in the case of dog's serum and rabbit's corpuscles and dog's serum and pig's corpuscles.

The fact that slight hæmolysis occurred in the control tubes to which sodium chloride had been added in place of rabbit's serum, after the rabbit's corpuscles had been treated at zero with dog's serum, is probably explained by a trace of hæmolytic serum remaining with the activated corpuscles even after careful washing.

That it is not due to bacterial action is shown by the fact that it did not occur in the control tube.

Incidentally I have been able to confirm the results of Hektoen on the inhibitory action of calcium chloride on hæmolysis, *in vitro*.¹ For instance, the addition of calcium chloride to defibrinated dog's blood (2 c.c. 0.5 per cent calcium chloride in 0.9 per cent sodium chloride, added to 8 c.c. of blood) arrested the hæmolytic activity of the serum for rabbit's washed corpuscles, ten minutes after the addition of the calcium chloride.

¹ HEKTOEN: Proceedings of the Chicago Pathological Society, 1903, v, p. 303.

IV.

Following a line of work suggested by Dr. G. N. Stewart,¹ experiments were undertaken to determine the effect on the production of hæmolysins and agglutinins of injecting corpuscles treated with calcium chloride (see Experiment 14).

Experiment 14. May 25. — 8.35 P. M. Added 0.5 gm. anhydrous calcium chloride to 10 c.c. dog's defibrinated blood, and put in ice-box.

May 26. 9.30 A. M. Removed the blood-calcium chloride mixture from the ice-box, and left at room-temperature until 11.00 A. M., when it was injected subcutaneously into Rabbit No. 2.

At the same time, injected Rabbit No. 1 with 10 c.c. untreated defibrinated blood from the same dog, and injected Rabbit No. 3 with 0.5 gm. anhydrous calcium chloride dissolved in 10 c.c. 0.9 per cent sodium chloride solution. All the rabbits weighed approximately 1500 gms.

The injections were made subcutaneously over the loins, 5 c.c. being injected on each side.

In a few days, sores developed at the points of injection in Nos. 2 and 3, and both rabbits gradually became emaciated. At the post-mortem examinations, the sores were found to extend through the skin and subcutaneous tissue into the muscles of their backs, and to be gradually healing. A calcareous deposit was present in the surrounding tissues.

June 24. — Made 10.0 per cent defibrinated dog's blood (S').

4.10 P. M. Put 0.6 c.c. of serum from each animal into correspondingly labelled tubes, and added 0.5 c.c. S' to each. Left at room-temperature.

4.12. 1, slightly hæmolysed; 2 and 3, no hæmolysis, but obviously agglutinated.

4.15. 1, strongly hæmolysed; 2 and 3, no hæmolysis, but strongly agglutinated.

4.23. 1, completely hæmolysed; 2 and 3, no hæmolysis; 2 is strongly agglutinated, but 3 more so.

7.05. 2 and 3, no hæmolysis; 2, less strongly agglutinated than 3.

4.12. A similar set of tubes was put up, only 0.1 c.c. of the sera were added to 0.5 c.c. of S', and put at 40° C. A control tube containing the same amount of S', to which 0.1 c.c. 0.9 per cent sodium chloride was added, was included in the set.

The following is a summary of the results:

Hæmolysis was slower in 1 than in the corresponding tube in the first set. No hæmolysis occurred in 2 and 3, or the control tube. After

¹ STEWART: This journal, 1904, xi, pp. 256-258.

three hours in the incubator, 0.1 c.c. dog's serum was added to 3; twenty-four minutes later, no obvious change had occurred in it.

Before hæmolysis began, the agglutination in 1 and 3 was about the same; *i.e.*, strong. The agglutination in 2 was never so strong as in 3. None occurred in the control tubes.

It was also shown that heating serum from Rabbit 3 to 56° C., for forty-five minutes, did not appreciably alter its agglutinating power for dog's corpuscles.

The corpuscles of Rabbit 1, which received the injection of untreated dog's blood, were readily hæmolysed by dog's serum.

For this purpose, a much larger quantity of anhydrous calcium chloride was added to dog's defibrinated blood than was necessary to abolish hæmolytic action, and allowed to act twelve hours in the ice-box, and then for two hours at room-temperature, before being injected into a rabbit. At the same time another rabbit was injected with the same amount of untreated blood, and another with the same amount of calcium chloride dissolved in 0.9 per cent sodium chloride solution. After a time, serum was obtained from each of the rabbits, and its action tested on dog's corpuscles.

Although this is the only experiment I have been able to make, the results were so definite as to be worth recording. As will be seen from Experiment 14, the mixing of calcium chloride with dog's blood before injecting into the rabbit destroyed, or greatly weakened, the hæmolysinogen, but not the agglutininogen.

The injection of calcium chloride alone, as might be expected, did not induce in the rabbit's serum a hæmolytic action for dog's corpuscles. The rabbit's serum, after injecting calcium chloride, had a marked agglutinating action on dog's corpuscles. Whether this was due to the calcium chloride or not, cannot be decided from one experiment. Thus calcium chloride appears to differ from formaldehyde in its action on the hæmolysinogen, as dog's blood treated with formaldehyde in amount sufficient to fix the corpuscles, still induces the production of a hæmolytic serum when injected into rabbits.¹

¹ STEWART: This journal, June, 1904, xi, pp. 256-258.



ON RHEOTROPISM. I.—RHEOTROPISM IN FISHES.

By E. P. LYON.

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THE usual theory regarding the mechanism of stimulation of organisms in currents of water or air is set forth in the following excerpts from the literature. Rádl, in his book on "Untersuchungen über den Phototropismus der Tiere," says concerning the ephemeridæ: "They react very sharply to a gentle current of air, turning the head toward the air current, but without leaving the place in which they are floating. The air current acts by exerting pressure on the body surface of the animals and evidently tends to draw the body into the direction taken by it. On the contrary, the fluttering wings strive to lift the body upward and forward. The points of application of this force, lifting the body, and the force of the air current need be only very slightly separated; and a force-couple will result, that will rotate the body until the two forces, the muscle force and the force of the air current, draw the body in opposite directions. . . .

"It is easy to pass from the rheotropism of the ephemeridæ to other analogous phenomena of rheotropism. It will everywhere be found that the pressure of the air current acts as one force and contraction of the muscles as the other."

Verworn in his "General Physiology" classes rheotropism, stereotropism, and geotropism together under the collective title barotaxis, since he believes that all alike are responses to pressure. "A second form of barotaxis," he says, "in which the stimulus is produced, not as in thigmotaxis by contact with a solid body, but by a gentle current of slowly flowing water, is rheotaxis, which was discovered by Schleicher and carefully investigated by Stahl ('84). This is the peculiarity belonging to certain organisms, of taking toward flowing water a direction of motion opposed to the direction of the current. Since these organisms thus turn toward a pressure-stimulus, rheotaxis is merely a special form of positive barotaxis."

Wheeler¹ has named the orientation of flying animals in the wind anemotropism, and thus expresses himself regarding the cause of the phenomenon: "It requires but a moment's consideration to see that anemotropism is only a special form of rheotropism. . . . The only difference lies in the fact that the insect reacts to a gaseous, the fish and myxomycete to a liquid current. In both cases the organism naturally assumes the position in which the pressure exerted on its surface is symmetrically distributed and can be overcome by a perfectly symmetrical action of the musculature of the right and left halves of the body."

It is noticeable that all these authors consider rheotropism a reaction to pressure. The pressure of the wind or water acting against one side of an animal is assumed to be a stimulus like light or migrating ions, leading to one-sided activity of such a kind that the animal at length receives the stimulus uniformly on both sides. Then a uniform activity of its symmetrically arranged contractile elements carries it forward in the stream. This is the general theory of the tropisms.

To us who stand on the earth, the pressure of the wind or water is a very real thing. But if we were floating in a fluid medium, it is doubtful if uniform currents would be known to us. To the aeronaut the earth seems to slip along beneath. All is still, and he feels no wind because his balloon has the same velocity as the air. In the dark he cannot tell whether he is moving or not. In a fog one has no sensation of being borne along by the tide. In other words, as is well known, we have no organ for the recognition of uniform motion, nor can we imagine how such an organ could act. Stimulation implies a change of relation between organism and environment. But if both in all their parts are moving at the same velocity, their relations do not change and the conditions for stimulation are wanting.

Or, look at the matter in a little different light: a fish swims forward in quiet water with a velocity of ten feet a second. Of course the head of the fish sustains a certain pressure or resistance. Now, suppose the fish to be in a uniform current having a velocity of ten feet per second. To hold its place in the stream the fish must put forth the same effort as would carry it forward ten feet per second in quiet water, and the pressure exerted on its head must

¹ WHEELER: *Archiv für Entwicklungsmechanik*, 1899, viii, p. 373.

be the same as if it were swimming at the same rate in a quiet lake. How can this pressure orient the fish in one case and not in the other?

Or, let us imagine the fish at rest in calm water. Its body sustains a hydrostatic pressure proportional to its depth below the surface. Now, imagine the fish at rest (*i. e.*, inactive) in a deep and uniform stream of water. It is carried along with the same velocity as the water. Its body is also sustaining hydrostatic pressure and no other; and if it is at the same depth as the other fish, this pressure is equal on the two fish. How can the pressure serve to orient in one case and not in the other? No fish or bird would advocate a pressure theory of rheotropism. But it came naturally enough from man, who stands upon the earth and has that as a reference point for every motion of water or air. To man the wind makes itself known as a pressure. But to the bird above the earth it must express itself in an apparent motion of the ground; and only when the bird attempts to keep its position with reference to the non-moving objects on the ground, does it begin to feel the pressure of the air current, and then only so much pressure as if it were flying in calm air with the same effort. Pressure results from orientation; it does not produce it.

It will be understood that in all these considerations I have in mind currents of uniform velocity. Gusty winds and irregular currents will need separate discussion.

It comes to this: Motion is a relative conception. It implies objects at rest as well as objects moving. We do not know that anything moves unless ourselves or other things are at rest. We are moving through space at a rate of hundreds of thousands of miles a day in the orbit of the earth around the sun. But all our ordinary reference points are going at the same rate; as a result, it took man unknown centuries to discover that he is always moving in this way. He never has any sensation of it. It is equally absurd to imagine a fish in the Gulf Stream to be stimulated and oriented by a uniform forward motion of the water. Whether orientation be a simple reflex or a conscious process, points of reference — *i. e.*, points relatively at rest — are necessary.

It was considerations like the foregoing which led me to believe that rheotropism is not a response to the current in the simple way that writers have assumed, but rather a response to relative motion. Bodies which do not move must be as important in bringing about

stimulation as those which do move, — indeed, more important in some cases. In the case of the fish in the stream, the bottom and banks evidently constitute a large part of the stationary environment. If these are of importance in determining the rheotropic responses, one thinks naturally first of the eyes as the organs through which the stimulation might take place. Furthermore, if rheotropism is a response to relative motion, we might expect the fish to be oriented as well if the water should stand still and the bottom should move (with reference to us and the fish), as in the usual case where the water moves and the bottom stands still. This turns out to be true.

Experiment 1. — An aquarium with a glass bottom was so supported that the bottom was freely accessible. Close along the bottom, beneath the glass, could be drawn a long piece of white cloth with black stripes painted across it. This would give the impression of a moving bottom. Fish (*Fundulus*) placed in the aquarium oriented themselves with the head in the direction of the moving bottom and swam along with it to the end of the aquarium. Reversing the movement of the bottom reversed the orientation and movement of the fish.

This experiment is striking enough to convince any one that orientation may come through the eye, and under conditions where no pressure changes occur. But not every fish responds (especially if we use adults); and we might think, therefore, that the eye, as part of the reflex arc, plays but a small part in rheotropism. It is to be noted, however, that the conditions are very artificial. The bottom is unnatural, the sides do not move, and the fish are disturbed by the presence of the observer.

It might seem at first glance, too, that the animals should move in the opposite direction to the moving bottom. But that is not the correct view, for in an ordinary current the fish tends to be borne downstream away from fixed points in the environment. In other words, we may say the bottom appears to be moving upstream, and the fish moves with the moving bottom.

Experiment 2. — A cylindrical glass dish about 12 cms. deep and 30 cms. across was supported from above by wires so as to remain stationary. Around this was placed a galvanized iron cylindrical dish about 18 cms. deep and 40 cms. diameter, supported from below so that it could be rotated about the common vertical axis of the two dishes and around the inner, stationary dish. The bottom of the rotating dish was covered with gravel, and some small seaweeds were attached to the sides. The inner vessel

and the space between the two were filled with water. A beaker of dark liquid was set in the middle of the inner cylinder, so that the fish could see only one side of the rotating cylinder at a time. Thus a circular pathway was formed in which the fish were placed. On turning the outside dish the fish moved round and round inside the stationary dish, and in the same direction as the moving environment.

Reversing the motion reversed the orientation.

With many species of young fish the experiment succeeds with clock-like regularity. With older fish, especially *Fundulus*, efforts to escape and to hide mar the result. Individual adults may perform well, but there are many exceptions. It is to be noted in this experiment also, however, that not all of the environment moves. As we shall see, cutaneous sensations, as touching the non-moving glass bottom, would tend to correct the illusion of a current. *Fundulus*, too, seem to learn quickly that they are being deceived. But by a modification of the experiment, suggested by Dr. Garrey, the importance of the optical stimulus even in the adult fish may be uniformly demonstrated. If one by stirring with the hand sets up a real current in the circular path occupied by the fish, they respond to it. Now, by rotating the outside dish opposite to the current, one may intensify the rheotropic response. By rotating it in the same direction as the current, one can annihilate the orientation.

Experiment 3. — The inner, stationary dish was removed, and the fish placed in the outer dish, the beaker being placed in the centre as before. In this case, even better than before, the fish went round and round, keeping with the moving bottom. Reversing the latter reversed the fish.

In this experiment, through friction between the container and the water, currents in the latter are actually set up. They are in the same direction as the rotating cylinder, and slower than the latter. Now, if it were the current *per se* which orients the animal, they ought to turn and go against the current in the cylinder. On the contrary, they move with the moving optical field, and therefore with and faster than the current. If, after revolving the cylinder several times and getting up a considerable current, the cylinder is suddenly stopped, the fish are borne on a short distance with the current, then turn and face it. But here the current does no more than passively carry the fish past the fixed points of the environment, and therefore create a relative motion of the latter opposite to that it had before (and this effect can be abolished or intensified by proper move-

ments of the cylinder). This, it seems to me, is all the current in the case of fish usually does. On placing fish in water I have noted repeatedly that they are first borne downstream a short distance, then turn and swim up. The stimulus is the moving or apparently moving optical field, and the current is only indirectly responsible.

This impression is very strongly borne upon the observer if young fish and shrimps (*Palemonetes*) are placed in the turn-table at the same time. On rotating the latter two sets of circulating organisms are seen. The fish swim round and round in the direction of the motion of the environment. The shrimps swim opposite to this, and therefore against the true current which the revolving cylinder sets up. On stopping the cylinder the fish turn, and both kinds of animals now face the current. I am not yet entirely ready to discuss the rheotropism of *Palemonetes*. (I hope to take up the rheotropism of the insects, crustacea, protozoa, etc., in a future paper.) But it appears in this experiment that there are two types of stimulation bringing about a rheotropic response, and in the fish apparently the optical type is the stronger. This is satisfactorily substantiated to my mind by the next experiment.

Experiment 4. — In a long bottle of about ten litres' capacity were placed some young fish, and the bottle entirely filled with water and corked. The bottle was submerged and held by wires lengthwise in the large open-air basin at the Fish Commission, near a wall covered with algae. Moving the bottle toward my right, the fish all crowded to the left-hand end. Moving the bottle toward the left, the fish all rushed to the right, and crowded up into the very neck of the bottle in dozens. Nothing could be more beautiful than the machine-like regularity with which a school of silver-side minnows responded. To make it still more striking, I buoyed up the bottle, and placed it in a shallow tide-stream. As I released it, and it started to float off downstream, every fish hastened to the upstream end of the bottle. Stopping the bottle, they swam about in all directions. Letting it go again, they were instantly oriented and swam to the up end. Pulling the bottle upstream against the current, they went to the downstream end.

This experiment seems to shut out all possibility of pressure effects in the orientation of these fish.

Finally, I will describe an experiment which is the simplest and yet perhaps the most convincing of all. It surely approaches natural conditions more closely than the others, unless it be the fourth experiment.

Experiment 5. — A box about 1.25 metres long, 50 cms. wide, and 35 cms. deep was made with board sides and bottom, but ends of coarse wire netting. The bottom was covered with gravel, and the sides with fucus and sea lettuce. The box was weighted so that its edge would be barely above water. The box was held lengthwise in a strong tide-stream. The *Fundulus* in it were beautifully oriented. Now the box was released and allowed to float away. Instantly the fish lost their orientation.

"But," one is inclined to say, "there is now no current in the box." That is true in the sense that both box and water now have the same velocity relative to the observer. But the focus of the idea is this: the relation (pressure, for example) between the water and the fish has not changed at any moment. If the orientation depended on some peculiar power on the fishes' part of responding to motion of water, they ought still to be oriented, for the motion of the water has not stopped nor changed. It was the stimulus of the box, of the fixed environment, which really caused the orientation. The current only tended to carry the fish passively away.

If we place the box in quiet water; the fish are of course unoriented. If we begin to draw the box through the water, immediately every fish swims in the direction of the motion of the box. Here again it looks as if we had a current through the box, but it is the latter which moves; the water stands still. The orientation cannot be due, therefore, to pressure, because the pressure relations have remained unchanged. Blind fish placed in the box do not respond as long as their bodies are in contact with water only.

I am therefore convinced that with the fishes experimented with (*Fundulus*, Scup, Stickleback, Butterfish) orientation in currents of fairly uniform velocity is usually an optical reflex. The current does not directly stimulate. Indirectly it does, by tending to move the fish away from the fixed points of its environment.

It remains to be determined whether the fish possesses other means of orientation besides that through the eyes. First, we may ask whether fish can orient themselves in the dark.

Experiment 6. — A trough was arranged with darkened labyrinths at each end, so that a current of water could be passed through it without admitting light. Some *Fundulus* were placed in it, and the box tightly closed. On suddenly admitting light, the fish were always found oriented and on the bottom.

Experiment 7.—Several *Fundulus* were blinded by enucleation. These fish, when put in the trough, through which a not too strong current was kept up, could be seen to swim about more or less at random until they touched the bottom; then they turned their heads against the current. Blind fish stay most of the time on the bottom. If fine sand is sprinkled over the bottom of the aquarium, the fish frequently drag themselves through with sufficient force to leave lines marking the contact of the ventral and anal fins with the bottom.

Experiment 8.—Some blind *Fundulus* were placed in the strong tideway leading into the Eel Pond. In this the water rushes with more or less eddy and irregularity. The fish, while up from the bottom, would occasionally head up. But on the whole they swam about irregularly (being carried steadily downstream) until they touched bottom. The instant they touched, they headed upstream. This was very striking; even slight and momentary contact with a spear of eel-grass or a tuft of sea lettuce gave the necessary stimulation and reference point, and the animal instantly oriented itself. Very quickly, however, it would be borne by the current away from its contact with the vegetation or other fixed object and would lose its orientation. Such fish were invariably carried out to sea.

It therefore appears that cutaneous sensations are able to effect orientation. But here again the solid, unmoving environment is what stimulates rather than the current itself.

The case is the same exactly as orientation through the optical reflex. Doubtless the two methods of stimulation are closely correlated and ordinarily act together. These observations make clear also why the fish in some of my turn-table experiments were able, by contact with the non-moving bottom, to correct the false optical impression of a current.

Dr. Parker¹ has shown that the lateral line nerves are not concerned in rheotropism, but rather the general cutaneous nerves. My experiments would suggest the probability that the ventral surface is especially concerned, since the fins and general surface of that side would touch solid objects most frequently. But I have made no experiments along this line.

There remains one other, at first sight, apparently different type of stimulation which may cause orientation of fishes in running water.

¹ Personally communicated.

Experiment 9. — A blind fish placed in a trough where water is gushing rather violently, for example, through a small hole, or through a glass tube, may orient itself without contact with solids and strive hard to swim against the stream.

This we might call a true rheotropism, as contrasted with the other forms which have been described. But I am convinced that the methods of stimulation are fundamentally alike. The condition in the trough is this: a high velocity in the middle, just where the stream enters, a less velocity next to the central stream, and even a negative velocity or back flow further to the sides of the trough. This relatively slower movement of part of the water is the essential element for stimulation, just as the relatively slower — *i. e.*, non-moving — bottom is the essential thing in the visual form of orientation. The fish could be seen to vary from side to side and to turn the tail and fins strongly, either into the more rapid water on one side or the slower stream on the other.

If one wishes in this case to attribute stimulation to differences in pressure, it is perhaps correct to do so. But the theory must be quite different from the gross mechanical one of Rádl. It must involve the idea of higher pressure on one part than on the other, through differences in velocity of the water striking the two parts. Through these differences of pressure orientation may be effected. It seems to me, however, rather more likely that the sliding contact between fish and water in the gushing stream is the stimulus, just as the sliding contact between fish and solids is able to effect orientation. Indeed, rheotropism in so far as it is a response to cutaneous stimulation has close relations with stereotropism.

Orientation of this kind would not be possible in a current of uniform velocity, for example, a wide and deep river. In shallow, swift streams it might in certain forms play an important part. It would be interesting, for example, to test trout as to the respective parts played by the optic and cutaneous nerves in orientation. Indeed, I saw indications that the sticklebacks were more sensitive to variations in the velocity of the water than *Fundulus*. Blind individuals of the former species were more easily oriented by a gushing stream when not in contact with the bottom. In all the fish with which I have experimented, however, I am convinced that the visual form of stimulation is by far the more important. Blind fish were not able to hold their places in streams where the normal ones were regularly found, and where there were visible differences in velocity. Even

by staying near the bottom they could not sufficiently and continuously orient themselves to prevent being borne down the stream. I could not attribute their failure to hold their places to muscular weakness, for they would swim against powerful streams in the laboratory, provided strong differences of velocity were produced in close proximity; and they remained alive for days. Moreover, the turn-table experiments already mentioned made clear that fish follow the moving visual field in preference to running against the coexistent and slower current, — a current amply able, however, to orient them, as could be seen by stopping the rotation. In a current formed by the rotation of a dish, evidently the swifter-moving layers would be those close to the bottom and walls of the cylinder. Next these would be slower and slower layers. Probably the shrimps which went against the current were oriented by these small differences of velocity, but the differences were not sufficient to orient the *Fundulus*, nor even, apparently, to render orientation through the eyes less precise.

A very good way of investigating rheotropism is that employed by Devitz.¹ Into a circular dish a stream of water from a rubber tube is introduced tangentially. Thus a current round and round the dish — an endless stream — is produced. The velocity varies, being greatest just where the water enters, and zero at the centre of the dish. On one occasion I had in such a dish two blind *Fundulus*, one of which persistently remained on the sandy bottom; the other, up in the water. With the former a series of trials was made, in the following way: the fish, having come to rest on a somewhat elevated central portion of the dish, was stimulated by means of a glass rod and caused to swim off toward the periphery and thus into the current. The record of a series of such trials follows: —

- 1st trial. — Swam radially, touched bottom, headed upstream.
- 2d trial. — Swam radially, touched bottom, headed upstream.
- 3d trial. — Swam radially, touched bottom, headed upstream.
- 4th trial. — Swam radially a short distance, turned downstream, swam once and a-half around, touched bottom, headed upstream.
- 5th trial. — Swam radially, touched bottom, headed upstream.
- 6th trial. — Swam radially, touched nose to side of dish, headed upstream.
- 7th trial. — Swam radially, touched bottom, headed upstream.
- 8th trial. — Swam radially, turned upstream, and sank to bottom.
- 9th trial. — Swam radially, touched bottom, headed upstream.

¹ DEVITZ: *Archiv für Physiologie*, Supplement-Band, 1899, p. 286.

- 10th trial. — Swam radially, touched bottom, headed upstream.
- 11th trial. — Swam radially, touched bottom, headed upstream.
- 12th trial. — Swam radially, touched side of dish, headed upstream.
- 13th trial. — Swam downstream half around, touched bottom, headed upstream.
- 14th trial. — Swam radially, touched bottom, headed upstream.
- 15th trial. — Swam radially, touched bottom, headed upstream.

Meanwhile, and for hours after, the other fish remained up in the water, occasionally touching the side and heading upstream for a moment, or partially orienting itself where the water current entered, but on the whole without orientation, and borne round and round the dish by the current. This fish was in good condition, and quite as active as the other specimen.

It may be well to point out here a relation which exists between rheotropism and the familiar behavior of animals on a turn-table. When revolved on an ordinary open turn-table, many animals run around in the direction opposite to the turning. In animals which do not have semicircular canals — insects, crustaceans — and in vertebrates whose eighth nerves have been severed, I have shown¹ that this compensatory motion is an eye reflex, and that it may be produced either by rotating the animal or the environment. The animal tends to follow the real or apparent movement of the surroundings. This is the case with a fish in a current of water. In my turn-table experiments with fish already described, the environment practically entire moved round, and the fish moved with it. Centrifugal force and semicircular canals were not concerned, for the water in which the fish were swimming was at rest, especially when an inner stationary dish was used. In streams where there is a real or apparent motion of the surroundings in a straight line, we may consider that we have a wheel of infinite diameter and therefore no centrifugal force. But the phenomenon remains the same, and I think we are justified in classifying rheotropic responses among compensatory motions. This must be true, it seems to me, whether the stimulus to orientation be optical or cutaneous.

The probable part played by rheotropism in the migration of fish may be worthy a moment's discussion. In wide and deep streams, where the velocity is fairly uniform, it seems that fish would only be oriented by sight of or contact with the bottom. Only in rushing

¹ LYON: This journal, 1899, iii, p. 86.

torrents would the difference in velocity of closely adjacent parts of the stream be sufficient to orient. It is to be noted further that an explanation of orientation, although a sufficient explanation for migration in the case of the other tropisms (the only additional factor being ordinary forward locomotion), does not in the case of rheotropism¹ explain migration. For here we have to do with a force which, unlike light, heat, or electricity, tends actually to transport the organisms passively from one place to another. Whether the fish stays in one place in the stream, goes upstream or downstream, depends on whether its activity (*i. e.*, velocity of swimming) equals, is greater, or is less than the current. In tide-streams fish ordinarily merely hold their places. Going upstream involves, beside orientation, a greater activity than that required to keep a place in the stream or retain a given optical field. What the stimulus to this increased activity is, we do not know.

SUMMARY AND CONCLUSIONS.

1. In the fish examined the primary cause of orientation in streams of some uniformity of motion is an optical reflex, a tendency on the part of the animals to follow the field of vision. The current tends to carry the fish downstream and therefore to cause a relative, opposite motion of the environment. To keep the same visual field, the fish moves against the stream. The essential element of stimulation is the environment, not the current. Any relative motion between the fish and its solid surroundings will stimulate and orient. The current is responsible for the orientation only in that it causes such relative motion between the fish and the bottom or banks of the stream.

2. Contact between the fish and stationary objects may lead to orientation. The conditions here are the same as in the preceding paragraph, the current playing only the passive part of sweeping the fish against objects on the bottom.

3. In violent streams where considerable differences of velocity exist in adjacent parts, the fish may be oriented without sight of or contact with solid objects. But here again relative velocities constitute the essential elements of stimulation. If part of the water moves, and that next to it is relatively at rest, the fish may respond just as it does to contact with solids.

4. In other organisms, also, it is believed that the rheotropic response must be brought about by one or more of the three methods of stimulation found in fishes.

5. Rheotropism in fishes is a form of compensatory motion.

6. Rheotropism explains the orientation, but not the migration of fishes.

THE IDENTITY OF SO-CALLED UREINE (MOOR).

By H. D. HASKINS.¹

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DR. WILLIAM OVID MOOR has recently stated that the true urea content of the urine is not over 50 per cent of the amount estimated by the best previous methods.² He believes that the over-estimation is due to another nitrogenous constituent which, while different in composition, has the same solubilities and the same chemical reactions as urea, and which therefore adds itself to the urea in the quantitative determination of the latter.³ This substance he calls ureine.

Moor describes ureine as an oily liquid belonging to the aromatic alcohols. On heating to 80° C., it changes into several bodies belonging to the class of aromatic oxyacids, and by further heating to 120° C. is reduced to pure carbon. Like morphine, it gives a blue color reaction with potassium ferricyanide and ferric chloride.

Dr. Moor's first work on ureine⁴ was criticised by W. J. Gies. The latter, following Moor's method, extracted ureine and found it to contain urea, kreatinin, pyrocatechin, purin bodies, phenol, alkaloidal substances, and inorganic salts sufficient to give an ash of 9 to 24 per cent.⁵

Dr. Moor further believed that he had discovered in ureine the cause of uræmia.⁶ Kuljabko investigated this point, and reported that the toxic symptoms following an injection of ureine closely re-

¹ H. M. Hanna Research Fellow, Western Reserve University.

² MOOR: *Zeitschrift für Biologie*, 1903, xlv, p. 123.

³ MOOR: *Loc. cit.*, xlv, p. 420.

⁴ MOOR: Medical record, 1900, lviii, pp. 336 and 471.

⁵ GIES: Medical record, 1901, lix, p. 329; also *Journal of the American Chemical Society*, 1903, xxv, p. 1295.

⁶ MOOR: *Le physiologiste russe*, 1900, ii, p. 128; abstract in *Biochemisches Centralblatt*, 1903, i, p. 386.

semble those following an injection of urine extractive substances,¹ he concludes further that ureine is not a simple body, but merely an alcoholic extract of urine, freed of part of its urea.

Moor's improved method of extraction, briefly stated, is as follows: urine is evaporated on a water-bath at 50° C., and the residue then re-dissolved in absolute alcohol, filtered and evaporated to constant weight. This final extract contains urea which crystallizes out and a yellow oily fluid or semifluid substance,—the ureine. The latter makes a spot on paper like a grease spot, which remains for a long time. It is very hygroscopic.

Moor shows that its fluidity cannot be due to urea, for urea is never hygroscopic. The possibility of ureine being identical with any of the known constituents of urine, he discusses briefly as follows: It cannot contain urine carbohydrates, because these are insoluble in absolute alcohol. It cannot be oxyproteic acid (discovered by Bondzynski and Gottlieb)² because this would be precipitated by barium hydroxide, whereas this reagent does not throw ureine out of solution. Traces of kreatinin, allantoin, or hippuric acid might go into the absolute alcohol extract, if present in the urine; but these bodies could not account for ureine, because they are crystalline and not hygroscopic.

The only other known constituent of urine that it might be is urochrome. Moor satisfies himself that he is not dealing with urochrome by the following line of argument: after treating a solution of ureine with silver nitrate or animal charcoal, he is able to recover the characteristic yellowish oily material; whereas these agents completely remove urochrome from a solution, and finally urochrome could not be present in his extract, because it is insoluble in absolute alcohol.

The present investigations go to show that the so-called ureine does contain urochrome, and that the above arguments are fallacious in every particular.

By some preliminary experiments, it was found that lead and barium compounds, as well as silver nitrate, did not remove all the colored material of urine, and this was found to be true even when all of these reagents were used successively on the same urine, although they did render the unconcentrated urine perfectly colorless to all appearance.

¹ KULJABKO: *Le physiologiste russe*, 1900, ii, p. 131; abstract in *Biochemisches Centralblatt*, 1903, i, p. 427.

² BONDZYNSKI and GOTTLIEB: *Centralblatt für die medicinischen Wissenschaften*, 1897, p. 578.

Experiment 1. — Urine was evaporated and extracted with absolute alcohol according to Moor's method. The residue was a brownish yellow semifluid substance. In a desiccator it became solid, containing a mass of urea crystals. On exposure to the air, it became semifluid, and the liquid part was drained off, effecting a mechanical separation from the urea. This ureine, so-called, was dissolved in absolute alcohol, and the solution filtered and evaporated. The resulting residue became solid in the desiccator, and showed no distinguishable crystals of urea. It was rubbed up with amyl alcohol, which removed a little urea and yellow material. It was then dissolved in water, and treated with an excess of silver nitrate which caused a precipitation. After precipitating the excess of silver, filtering, and evaporating, a residue was obtained which was yellow in color.

This experiment cast doubt on Moor's assurance that urochrome could not be present in an absolute alcohol extract. We also find in Neubauer and Vogel¹ the statement that urochrome is soluble in absolute alcohol. Not only was the presence of urochrome indicated as probable by the silver nitrate precipitate, but also the color of the final residue seemed to indicate a possible incompleteness of precipitation.

Experiment 2. — In this experiment the urine residue was extracted with 95 per cent alcohol. Alcoholic silver nitrate was added to excess. The excess of silver was removed. The final filtrate was evaporated at a low temperature. The residue contained urea crystals and a yellow substance. This was treated with several portions of chloroform containing 10 per cent of absolute alcohol, the residue was transferred to the filter, drained, and finally pressed between filter paper. This effected a separation from the urea, for the latter was left on the surface as almost colorless crystals, while the yellow material soaked into the paper. The urea was brushed off and the torn pieces of filter paper were shaken with chloroform-alcohol mixture, completely removing the color.

The fact that chloroform containing alcohol is a good solvent for urochrome added to the suspicion that there might be urochrome in the so-called ureine, and that silver nitrate might not always be efficient in removing urochrome.

Experiment 3. — The residue from the chloroform extract of the preceding experiment, and also some of the so-called ureine freshly prepared according to Moor's directions, were tested as follows to determine whether they would respond to tests mentioned by Moor, and to those given by urochrome.

¹ NEUBAUER and VOGEL: *Analyse des Harns*. Analytischer Theil, 1898, p. 505.

1. Ureine (?) solution turns blue, then green, when potassium ferricyanide and ferric chloride are added.
2. Ureine (?) turns Fehling's solution green; on heating, brown.
3. Ureine (?) removes the pink color from a dilute solution of potassium permanganate.
4. Ureine (?) gives off acid fumes, and leaves a black char when heated in a crucible.

These agree with Moor's findings.

Various tests applicable to urochrome were then tried.¹

5. Solubilities: soluble in absolute alcohol, acetic ether, and amyl alcohol; slightly soluble in acetone, ether, and chloroform (in the two last probably because of the presence of moisture on account of the deliquescent condition of the ureine), more readily soluble in ether or chloroform containing some alcohol.
6. On adding hydrochloric acid and heating, ureine turns brownish and leaves a brownish black residue.
7. Nitric acid gives a reddish color with purple margins, but no waxy mass.
8. Ureine burnt in a crucible leaves practically no ash.
9. A deeply colored alcoholic solution causes no absorption bands to appear, but a diffuse absorption of the violet end of the spectrum.
10. Ureine solution gives a precipitate with silver nitrate.
11. Ureine solution gives a precipitate with lead acetate.
12. Ureine solution gives the xanthoproteic reaction.
13. Ureine solution saturated while warm with ammonium sulphate, and shaken with alcohol, causes the separation of an orange-colored top layer of alcohol.
14. Ureine solution mixed with a great excess of animal charcoal, and kept at 40° C. over night gives up a slight amount of yellow material to alcohol.
15. Ureine solution added to a strong solution of uric acid in alkali, and the uric acid precipitated by hydrochloric acid, gives a brownish red deposit, showing under the microscope colored crystals of uric acid just as they appear when precipitated from urine.
16. Ureine mixed with an excess of alcoholic oxalic acid and filtered gives a yellow solution which after removal of the excess of oxalic acid gives the xanthoproteic reaction.

Tests 7 and 16 were suggested by Moor's statement that oxalic and nitric acids form insoluble compounds with ureine, and the results

¹ NEUBAUER and VOGEL: *Loc. cit.*, pp. 504-507.

tend to controvert that statement. Probably the presence of considerable urea nitrate or oxalate misled Moor, as Gies suggested.

Experiment 4. — Urochrome was prepared according to A. E. Garrod's method.¹ This urochrome resembled ureine closely in color and odor, and further in being amorphous and very hygroscopic. Tests 1–16 of the previous experiment were applied with identical results.

A comparative test was made on urochrome and ureine to see whether silver nitrate and animal charcoal could remove all the colored material.

(a) A concentrated solution of urochrome and also of ureine was made, to each was added silver nitrate solution to excess, the excess of silver was precipitated and the solution was shaken down with the centrifuge. The yellow supernatant fluid was saturated with ammonium sulphate and shaken with alcohol. The separated alcohol was strongly colored yellow, and gave the xanthoproteic reaction.

(b) About the same quantities of urochrome and ureine solutions of about the same concentration were treated with the same amount of animal charcoal, shaken up, corked, and left in an oven at 40° C. over night. On shaking down, the charcoal occupied more than half of the bulk of the material. The clear supernatant fluid was slightly yellow in each tube, and after concentrating, each gave a distinct xanthoproteic reaction.

Therefore urochrome and ureine are affected in the same way by silver nitrate and charcoal, and neither is completely removed by these agents.

CONCLUSION.

Moor's ureine is a mixture containing a large amount of urochrome. The reactions which Moor believes establish the existence of his ureine are in reality the characteristic reactions of urochrome.

¹ GARROD: Proceedings of the Royal Society, 1894, lv, p. 394; also NEUBAUER and VOGEL, *Loc. cit.*, pp. 506–507.

THE CHEMISTRY OF MALIGNANT GROWTHS. II.— THE INORGANIC CONSTITUENTS OF TUMORS.

By S. P. BEEBE.

[Contributions from the Huntington Fund for Cancer Research, Loomis Laboratory,
New York.]

IT is now well known that the small quantities of the inorganic elements which occur in the tissues of plants and animals are essential to the life activities of the organism. The old experiments of Forster¹ demonstrated conclusively that these substances fill quite as important a function in maintaining the life of an animal as the very complex proteids and carbohydrates in the food. Recent work has shown that the function of these inorganic elements is specific and that one element cannot be replaced physiologically by another, however close the chemical resemblance of the two may be. Bokorny,² for instance, tried to replace the potassium, in a medium on which certain moulds were grown, with rubidium, lithium, and caesium. He found that this could not be done, and we must conclude from all the evidence in these and the careful experiments of Herbst,³ that the smallest chemical difference is sufficient to give an element an entirely different physiological rôle.

The work done by Loeb⁴ and his co-workers during the last few years has revolutionized our ideas regarding many fundamental physiological processes, and has demonstrated in how great a degree vital activities are dependent upon the physico-chemical environment, — an environment profoundly influenced by the nature and amount of the inorganic salts.

¹ FORSTER: *Zeitschrift für Biologie*, 1873, ix, p. 297.

² TH. BOKORNY: *Archiv für die gesammte Physiologie*, 1901, xcvi, p. 134.

³ C. HERBST: *Archiv für Entwicklungsmechanik*, 1895, ii, p. 455; 1897, v, p. 649; 1900, ix, p. 424; 1901, xi, p. 617.

⁴ LOEB: a large portion of Loeb's work has appeared in this journal during the years 1900-1903. It seems unnecessary to give each page.

The particular function which such an element as calcium has in a secreting gland or in muscle tissue is not perfectly plain. It is probable that these soluble, easily diffusible salts have much to do with maintaining the proper osmotic environment of a cell during the continuous and varied changes in the surrounding medium. They are, therefore, important aids in the nutritional processes of a tissue.

For these reasons it has seemed advisable to make analyses of the ash of tissues from malignant growths, to determine, if possible, whether a peculiar inorganic environment may have anything to do with the remarkable nutritional activities characteristic of neoplasms. It is true that ash analyses do not show the conditions in the living tissues; the metals may be present in the cell as ion-proteid¹ combinations, but the amount of calcium relative to potassium or sodium, for instance, can be determined by such an analysis, and recent work has demonstrated that the relative proportion of these elements may determine the degree of activity in such important structures as glands and muscles.

In this paper are given the results obtained from the ash analysis of nine neoplasms, eight malignant and one benign. Seven of these were removed at operations, the remaining two being obtained post mortem. The following elements were determined: Sulphur, phosphorus, nitrogen, iron, calcium, sodium, and potassium. Magnesium is undoubtedly of considerable importance; but the quantity found was so exceedingly small that the material at my disposal did not permit satisfactory determinations of this element to be made. In no case was a smaller quantity than 5 gms. of dried tissue used for ashing, and the final product in the determination of calcium, potassium, and sodium represented the amount of these metals found in 2 gms. of the original tissue. In all but one instance (carcinoma 9, primary growth in the pancreas) the analyses were made in duplicate.

The methods used for each element were as follows: for sulphur, the usual fusion with sodium hydroxide and potassium nitrate, the sulphur being finally weighed as barium sulphate; for phosphorus, Neumann's² alkali method; for nitrogen, the Kjeldahl method. In determining the calcium, potassium, and sodium, the dried tissue was ashed in a porcelain crucible at a dull red heat. Calcium was precipitated as the oxalate, and weighed as the oxide; the sodium and

¹ LOEB: This journal, 1900, iii, p. 327.

² NEUMANN: Zeitschrift für physiologische Chemie, 1902-3, xxxvii, p. 129.

potassium were weighed together as sulphates, the potassium then precipitated as the chlorplatinide, and the sodium obtained by difference.

For the iron, a colorimetric method was used, and the details of the method being in this case original, it has seemed advisable to give them.

METHOD FOR COLORIMETRIC DETERMINATION OF IRON.

1. A solution of ferric iron containing $\frac{1}{100}$ of a milligram of iron per cubic centimetre. This is most conveniently made by dissolving 0.0700 pure ammonia ferrous alum in a small quantity of water, adding 1 c.c. of dilute sulphuric acid and 1 c.c. hydrogen peroxide. Boil until the excess of peroxide is driven off; cool and dilute to one litre.

2. A 30 per cent solution of ammonium sulphocyanide.

3. An acid solution containing 10 per cent hydrochloric acid, and 2 per cent sulphuric acid.

Procedure. — One-half gram of the dried tissue is ashed in a platinum crucible. After cooling, the crucible is placed in a beaker of boiling water to which has been added 10 c.c. of the acid mixture. The iron is oxidized by means of peroxide, the solution filtered, cooled, and diluted to 250 c.c. To 50 c.c. of this solution in a long Nessler tube are added 2 c.c. of the ammonium sulphocyanide solution, and the two thoroughly mixed. The color thus obtained is compared with a control tube to which a known quantity of iron has been added.

The writer has found this method rapid and accurate. Tested with known solutions of iron, this method gave results on three trials having errors of 1.6 per cent, 1.8 per cent, and 1.7 per cent, respectively.

Preparation of the tissues. — Great care was taken to use only the pure cancer tissue for analysis. This was thoroughly ground in a hashing machine, and then dried in an oven, using at first a low degree of heat, 60°, and gradually raising the temperature to 100°, where it was maintained for at least thirty-six hours. The dried tissue was ground to a fine powder which could be kept indefinitely. In only one instance, the hypernephroma, was the fat extracted prior to the analysis, and in order to secure uniformity, the results in this case have been calculated on the weight of the tissue *before the extraction*.

RESULTS OF THE ANALYSES.

One hundred grams of the dried tissues contain the given weight — in grams — of the several substances.

Type of tumor.	N	S	P	Fe	CaO	K	Na
1. Carcinoma of the stomach	11.312	0.709	1.015	0.028	0.100	0.8900	0.8550
2. Carcinoma of the liver	10.860	0.771	0.680	0.064	1.200	0.5185	0.8550
3. Uterine fibroid	14.560	0.981	0.139	0.013	0.120	0.4400	1.1150
4. Hypernephroma	9.337	1.380	0.550	0.035	0.1130	0.1328	0.4765
5. Sarcoma (17)	10.860	0.755	0.940	0.054	1.1440	0.0800	0.7200
6. Sarcoma (18)	11.640	0.657	1.005	0.025	0.0900	1.6250	3.3150
7. Sarcoma (23)	12.200	1.122	1.060	..	0.1100	1.1180	1.9850
8. Epithelioma of the cervix	11.870	0.768	0.920	..	0.1470	0.6400	1.0875
9. Carcinoma of the liver —							
<i>a.</i> Primary in pancreas	0.1420	0.3831	1.0155
<i>b.</i> Secondary in liver	11.760	0.753	0.590	..	0.1150	0.9740	1.3435
<i>c.</i> Liver tissue	11.760	0.823	1.000	..	0.0700	0.8265	1.2720

DISCUSSION OF RESULTS.

Nitrogen. — The difference in the percentages of nitrogen is probably to be explained by the variations in the amount of fat contained in the tissue.

The lowest figure, 9.33 per cent, was from a tissue, the hypernephroma, having 29 per cent of ether-soluble material; while the highest, 14.56 per cent, was a tissue, the uterine fibroid, having only a small quantity of fat. The figures for the remaining tumors are quite constant. It is interesting to note that in carcinoma No. 9, the nitrogen in the secondary growth was precisely the same as in the normal liver tissue surrounding it.

Sulphur. — The most noticeable fact about the sulphur, as shown by these results, is the uniformity except in the case of the hypernephroma. In this growth the ratio of sulphur to nitrogen is much higher than in the other cases, indicating the presence of a compound comparatively rich in this element.

Phosphorus. — A portion of this element comes from the nucleoproteid of the tissue. The growth having the smallest amount of nuclear material, the uterine fibroid, has likewise the smallest amount of phosphorus.

Iron. — A portion of the iron undoubtedly comes from the blood remaining in the tissue. Another portion probably comes from the nuclein.

The tissue poorest in phosphorus is also poorest in iron. The two tumors having the highest content of iron have the highest content of calcium, but there appears to be no definite ratio between these two elements.

Calcium, potassium, and sodium. — The ratio which these metals have to one another, as shown by these analyses, is in many ways the most interesting result of the work. The carcinoma of the liver, No. 2, obtained at an autopsy, and sarcoma No. 5, removed at an operation, were badly degenerated. The conditions found in this sarcoma as a result of autolysis have been reported in a previous paper.¹ The carcinoma of the liver was not examined chemically for autolytic products, but inspection and histological examination showed that it was much degenerated. These two growths contained about ten times the amount of calcium found in the fresh vigorously growing tumors, and there was at the same time a much smaller content of potassium. The figures for the sodium are not so striking, but show a smaller content of this metal than was found in the fresh specimens.

The contrast between sarcoma No. 5 and sarcoma No. 6 is especially marked. Chemical examination of sarcoma No. 6 was mentioned in a previous paper.¹ One (sarcoma 5), a greatly degenerated tumor, contained a relatively large amount of calcium, 1.114 per cent, and a small amount of potassium, 0.06 per cent; the other (sarcoma 6), a rapidly growing fresh tumor of the same type, contained a small amount of calcium, 0.09 per cent, and a large amount of potassium, 1.62 per cent, and sodium, 3.315 per cent.

The hypernephroma, also, was much degenerated (see previous paper),¹ but although the potassium and sodium are low, there is no such increase in the calcium as seen in the other degenerated growths, Nos. 2 and 5.

Tumors 1, 3, 6, 7, 8, and 9 were fresh tissues growing rapidly. Tumors 2, 4, and 5 were degenerated growths. The contrast between

¹ BEEBE: This journal, 1904, xi, p. 139.

the relations of calcium to potassium and sodium in the two groups is quite as marked as the histological appearance. The analyses indicate that degeneration is accompanied by an increase in calcium or decrease in potassium; the ratio between the two is altered by a relative increase in the calcium. The conditions found in tumor No. 9 are interesting as affording some idea of this change. This carcinoma, obtained post mortem, was primary in the tail of the pancreas with metastases in the liver. The analyses show that the primary growth, which was somewhat degenerated, but not to the extent of tumors 2, 4, and 5, was poorer in potassium and sodium, and richer in calcium than the secondary growths in the liver.

It is impossible from so few experiments to draw definite conclusions as to the function of these metals or, indeed, from any number of purely analytical results, but the conditions found appear to be in accord with what is known regarding the physiological rôle of calcium and potassium. In their effects on vital phenomena, they stand opposed. The intense activity brought about by the potassium ion in the gland cells of the intestine¹ is stopped by the calcium ion. Their effects on muscle tissue, also, although the precise method of the action is in dispute, is plainly an antagonistic one.

In the degenerated tissues the vital activity has ceased, only autolysis is going on, and in these tissues is found the large amount of calcium. The fresh, vigorous tissues, having active nutritive changes, contain a relatively large amount of the ion which has the power to call forth vital activity. There is no reason to suppose that the potassium ion by its presence has acted as the catalyzer to cause the rapid cell division in these neoplasms; but its presence does seem to be in some way associated with their remarkable nutritional activities, of which rapid growth is one manifestation.

The relations between cause and effect are as yet too obscure to form the basis for any theories, but it would be interesting to know whether, as in the gland cells of the intestine, the continued exposure of the cells in a rapidly growing tumor to the influence of the calcium ion would be followed by an inhibition of the intense metabolic activity associated with the preponderance of the potassium ion.

It is the writer's intention to make a further study of this subject.

¹ MACCALLUM: University of California publications, *Physiology*, 1903, i, p. 5; this journal, 1904, x, p. 251.

INHIBITION OF THE ACTION OF PHYSOSTIGMIN BY CALCIUM CHLORIDE.

BY SAMUEL A. MATTHEWS AND ORVILLE H. BROWN.¹

[From the Hull Physiological Laboratory of the University of Chicago.]

IT has been shown by Loeb² that certain electrolytes, as sodium citrate, tartrate, oxalate, phosphate, and barium chloride, when injected into the lymph spaces of the frog, produce marked muscular twitchings. In fact, there is a general hypersensitiveness of the muscular and nervous systems. If at the same time or shortly after the injection of any one of the above-mentioned salts, in sufficient quantities just to produce tremors, there is given a proper dose of calcium chloride or strontium chloride solution, the tremors and tetanus do not appear.

It has also been observed that many of these same salts which produce an increased hypersensitiveness in the frog have a similar action on mammals. The additional observation has been made on the latter, that there are produced marked peristaltic contractions of the intestine, and a transient glycosuria. MacCallum³ has shown that the peristalsis so produced can be counteracted or prevented by the injection of calcium chloride. Fisher,⁴ at the University of California, and Brown,⁵ at the University of Chicago, showed that the glycosuria produced by electrolytes was inhibited by a sufficient amount of calcium chloride in the solution that would otherwise produce it. Brown showed also that strontium chloride had an action similar to the action of the calcium chloride.

It is well known that there is an antagonism between the action of certain alkaloids, as between atropin and pilocarpin, atropin and

¹ At the St. Louis University.

² LOEB: The decennial publications, the University of Chicago Press, 1902, x.

³ MACCALLUM: University of California publications, Physiology, 1904, i, pp. 175-197.

⁴ FISHER: University of California publications, Physiology, 1903, i, pp. 77-79.

⁵ BROWN: This journal, 1904, x, pp. 378-381.

nicotin, and atropin and physostigmin. It is reasonable to presume that calcium chloride, since it inhibits the tremors produced by sodium citrate and other salts, would have also a counteracting effect upon strychnine tetanus. But Fisher¹ has shown that such is not the case. S. A. Matthews² has shown that the convulsions produced in tetanus are inhibited by intravenous injections of a combined salt solution in which calcium chloride is present.

The object of this paper is to show that calcium chloride inhibits, in part at least, the action of physostigmin upon dogs. The action of this alkaloid, even in a minute dose, is to produce an increased peristalsis of the intestine, whether applied locally or injected into the circulation directly. Feeble muscular tremors may also result. In larger doses the bowel becomes contracted, hard, and pale. A general muscular tremor ensues, and may develop into general muscular spasms. The secretions, especially those under the control of the nervous system, notably the salivary secretion, are greatly increased. The heart-action and respiration are greatly slowed, and when a large dose is administered, it is necessary to use artificial respiration.

Medium-sized dogs were used for the experiments. The citation of the results of one experiment which did not differ in any important way from any one of the number performed, will suffice. A dog of about four kilos was anesthetized with two grams of chloretone. Cannulas were put into the trachea, the submaxillary duct, and the femoral vein; and a loop of the intestine was exposed by a median incision through the abdominal wall. Ten milligrams of physostigmin in an $\frac{m}{8}$ solution of sodium chloride were injected into the circulation through the femoral vein. Within half a minute the intestines were contracted so that they were small and pale and hard. In a few minutes, tremors of the muscles here and there over the body and limbs began, and soon became general. A few minutes later, five milligrams more of the alkaloid were injected as before. Further contraction of the intestine could not be discerned, but there was a notable increase in the intensity and extent of the tremors of the voluntary muscles. The saliva, in the mean time, was flowing from the cannula in the submaxillary duct, at a rate of about twelve drops per minute.

¹ FISHER: This journal, 1904, x, pp. 345-350.

² S. A. MATTHEWS: Journal of the American Medical Association, 1903, xli, p. 565.

Ten minutes after the injection of the last dose of physostigmin, an injection of 20 c.c. of an $\frac{m}{8}$ solution of calcium chloride was begun and allowed to flow in at the rate of 1 c.c. per minute. After the first three or four minutes there was a noticeable decrease in the contraction of the intestine and the tremors of the muscle; and by the time the 20 c.c. were in the circulation, the intestines were perfectly relaxed, and of normal consistency and color, and the muscular tremors had entirely subsided. The rate of the salivary secretion had decreased to five drops per minute.

In the next fifteen minutes, forty milligrams of physostigmin were given and there was no apparent effect. The intestine did not contract, there were no muscular tremors, and the flow of saliva was not accelerated. A local application of the physostigmin to the intestine and to the muscle was also made without producing a contraction of either.

In all the experiments, artificial respiration was used. It was not observed that the heart was any way affected by the calcium chloride. Further experiments will be made to study the antagonism between calcium chloride and this alkaloid, and others closely related in action.

CONCLUSIONS.

The action of physostigmin in producing (1), the contractions of the intestine, and (2), the tremors of the voluntary muscles, is counteracted and inhibited by calcium chloride; (3), the increased salivary secretion is, at least, partly counteracted and inhibited by the calcium chloride.

ON THE ORIGIN AND PRECURSORS OF URINARY INDICAN.¹

By FRANK P. UNDERHILL.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

SINCE the work of Jaffé,² Salkowski,³ and others,⁴ it has been generally recognized that the formation of indol in the animal organism is due to putrefactive processes in the intestine. According to Jaffé, indol is the mother-substance of urinary indican. In recent years the study of this constituent of the urine has received much attention, because it has been indicated that indol may arise in the body from processes other than those of putrefaction. Thus the observations of Blumenthal and Rosenfeld⁵ have shown that fasting rabbits may eliminate urine rich in indican, while not a trace of indol can be detected in the intestine. Furthermore, when rabbits are poisoned with phlorhidzin,⁶ to the extent of causing tissue decomposition, sufficient to induce an increased output of nitrogen in the urine, there is also an accompanying increased elimination of indican. Phlorhidzin is not peculiar in this respect; for, according to Harnack and v. d. Leyen,⁷ similar results may be obtained if animals are poisoned with oxalic or sulphuric acids. Blumenthal and Rosenfeld have, therefore, put forth the view that the indican of the urine may arise from tissue-

¹ An account of some of these experiments was presented to the American Physiological Society, December, 1903. (See Proceedings of the American Physiological Society. This journal, 1904, x, p. xxvii.)

² JAFFÉ: *Centralblatt für die medicinischen Wissenschaften*, 1872, i, p. 2; also *Archiv für pathologische Anatomie*, 1877, lxx, p. 72.

³ SALKOWSKI: *Berichte der deutschen chemischen Gesellschaft*, 1876, ix, p. 138.

⁴ SCHOLZ: *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 513.

⁵ BLUMENTHAL and ROSENFELD: *Charité-Annalen*, 1903, p. 46.

⁶ BLUMENTHAL and ROSENFELD: *Loc. cit.*; also LEWIN: *Beiträge zur chemischen Physiologie und Pathologie*, 1902, i, p. 472.

⁷ HARNACK and v. d. LEYEN: *Zeitschrift für physiologische Chemie*, 1900, xxix, p. 205.

decomposition within the organism, entirely independent of the putrefactive processes of the intestine; and since indol has not been found elsewhere in the tissues of such experimental animals, they have sought for a precursor of indican other than indol.

On the other hand, Scholz¹ has shown by experimental observations on animals subjected to the effects of oxalic acid and phlorhizin administration that there is as yet insufficient evidence that indican may arise from any source other than indol formed by putrefaction in the intestine. Ellinger² has repeated the work of Blumenthal and Rosenfeld, and finds that if fasting rabbits are prevented from eating their *fæces*, the indican of the urine will either be greatly diminished or entirely disappear. These results are in entire conformity with those obtained upon the fasting cat,³ dog,⁴ and man,⁵ and are opposed to the view that the fasting rabbit differs from other animals.⁶ Since, in the work of Blumenthal and Rosenfeld, great significance has been laid upon the fact that no indol could be detected in the intestinal contents of fasting rabbits, Ellinger,⁷ upon turning his attention to this point, has demonstrated that the method employed by Blumenthal and Rosenfeld for the detection of indol was not of such delicacy as to show traces of indol. On account of its ready absorption, the quantity of indol in the intestinal contents at any given time may be too small to be detected by the method of Blumenthal and Rosenfeld. Thus the preponderance of evidence as to the origin of indican seems to be in favor of the older, generally accepted, theory maintaining putrefaction as a cause.

The more recent experiments of Ellinger and Gentzen⁸ have demonstrated that tryptophan may be one, or the only, precursor of indol (and indican). If indol is given to indican-free rabbits, either subcutaneously or *per os*, the animals remain indican-free, but if the indol be injected into the cæcum, the urine subsequently eliminated is very rich in indican. The constitution of tryptophan has been indicated

¹ SCHOLZ: *Loc. cit.*

² ELLINGER: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 44.

³ MÜLLER: *MALY'S Jahresbericht über die Fortschritte der Thier-Chemie*, 1886, xvi, p. 210.

⁴ KRAUSS: *Zeitschrift für physiologische Chemie*, 1894, xviii, p. 167.

⁵ MÜLLER: *Berliner klinische Wochenschrift*, 1887, No. 24, p. 433.

⁶ BLUMENTHAL and ROSENFELD: *Loc. cit.*

⁷ ELLINGER: *Loc. cit.*

⁸ ELLINGER and GENTZEN: *Beiträge zur chemischen Physiologie und Pathologie*, 1903, iv, p. 171.

by Hopkins and Cole,¹ and later revised by Ellinger,² who has also shown its intimate relation to kynurenic acid. Hopkins and Cole³ have likewise pointed out that the Adamkiewicz (glyoxylic acid) reaction of proteids is attributable to the presence of the tryptophan group in the latter. Various proteid substances yield the Adamkiewicz reaction with different degrees of intensity—in some cases with entirely negative outcome. Of the substances that fail to give the reaction gelatine is the most familiar. In this connection it is of interest to note that Nencki⁴ long since was unable to obtain indol among the putrefactive products of gelatine.

EXPERIMENTAL.

As gelatine does not contain the tryptophan group, it ought, when made the chief nitrogenous constituent of the diet, to cause a diminished excretion of indican in the urine. An experimental study, the details of which follow, has been made to test the validity of this hypothesis.⁵

The general plan of the experiments was to feed dogs upon a mixed diet of which the chief nitrogenous constituent was some proteid food which will yield a large output of indican. Lean meat is well known to have such an influence.⁶ The meat period was followed by a gelatine period, and this in turn by an after-period with meat. Owing to the fact that after a meat period several days might elapse before one could be sure that the influence of the meat diet with its continued large output of indican, had disappeared, several experiments were carried out in which there was first a cracker-dust period, followed by a gelatine period, and finally a meat period. The gelatine fed was commercial gelatine, giving no Adamkiewicz reaction (Hopkins' modification with glyoxylic acid). The animals received the same quantity

¹ HOPKINS and COLE: *Journal of physiology*, 1903, xxix, p. 451.

² ELLINGER: *Berichte der deutschen chemischen Gesellschaft*, 1904, xxxvii, p. 1801.

³ HOPKINS and COLE: *Journal of physiology*, 1902, xxvii, p. 418.

⁴ NENCKI: *MALY'S Jahresbericht über die Fortschritte der Thier-Chemie*, 1877, vi, p. 31.

⁵ The only experiments on this point known to the writer are those carried out by SALKOWSKI (*Loc. cit.*), who found that on a three days' period of gelatine-feeding a dog gave out 3 mgms. of indican against 5 mgms. on a meat diet.

⁶ ELLINGER and PRUTZ: *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 399.

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of nitrogen in both the meat and gelatine periods. They were catheterized daily. The fæces were not analyzed. The nitrogen of the urine was estimated by the Kjeldahl-Gunning method, and the indican by the method of Ellinger.¹ In the following tables the correction proposed for more exact results with this method has been made.

EXPERIMENT 1.

MEAT PERIOD.								
Date, 1903.	Weight.	FOOD.	Nitro- gen of food.	URINE.				Remarks.
				Vol.	Sp. gr.	Nitro- gen.	Indigo.	
Nov. 3	kilos 4.4	grams { Cracker-dust 50 }	grams 6.12	c.c. 175	1.027	grams 5.24	mgms. 5.88	No defæ- cation.
" 4	4.4	{ Meat . . 150 }		200	1.024	5.36	7.60	No defæ- cation.
" 5	4.4	{ Lard . . 25 }		190	1.027	5.28	5.82	Defæca- tion.
" 6	4.4	{ Water . . 140 }		201	1.025	5.25	8.76	Defæca- tion.
GELATINE PERIOD.								
" 7	4.4	{ Cracker-dust 50 Gelatin . . 38 Lard . . 25 Water . . 140 }	6.12	156	1.036	7.12	8.20	No defæ- cation.
" 8	4.4			170	1.032	6.59	4.40	Defæca- tion.
" 9	4.4			136	1.038	6.24	2.80	Defæca- tion.
" 10	4.2			150	1.035	6.12	3.20	Defæca- tion.
" 11	4.2			130	1.040	6.62	2.80	Defæca- tion.
" 12	4.2			174	1.030	6.23	4.40	Defæca- tion.
" 13	4.2			150	1.032	5.69	4.20	Defæca- tion.
MEAT PERIOD.								
" 14	4.2	{ As in fore- period }	6.12	150	1.025	4.52	7.60	Defæca- tion.
" 15	4.4			195	1.022	4.79	8.00	Defæca- tion.
" 16	4.4			190	1.023	4.07	10.00	Defæca- tion.

¹ ELLINGER: *Ibid.*, 1903, xxxviii, p. 178.

EXPERIMENT 2.

CRACKER-DUST PERIOD.								
Date, 1903.	Weight.	FOOD.	Nitro- gen of food.	URINE.				Remarks.
				Vol.	Sp. gr.	Nitro- gen.	Indigo.	
Nov. 19	kilos 4.4	<small>GRAMS</small> { Cracker-dust 100 }	<small>GRAMS</small>	<small>c.c.</small> 140	1.025	<small>GRAMS</small> 1.49	<small>MGMS.</small> 4.20	No defæca- tion.
" 20	4.2	{ Lard . . . 25 }	1.72	150	1.012	1.63	2.00	No defæca- tion.
" 21	4.2	{ Water . . . 140 }		138	1.015	1.40	2.40	Defæcation.
GELATINE PERIOD.								
" 22	4.2	{ Cracker-dust 50 Gelatine . . . 38 }	6.12	170	1.030	6.66	1.40	Defæcation.
" 23	4.4	{ Lard . . . 25 Water . . . 140 }		140	1.040	6.76	1.96	Defæcation.
MEAT PERIOD.								
" 24	4.4	{ Cracker-dust 50 Meat . . . 150 Lard . . . 25 Water . . . 140 }	6.12	60	1.035	2.30	5.50	Defæcation.

EXPERIMENT 3.

CRACKER-DUST PERIOD.								
Date, 1903.	Weight.	Food.	Nitro- gen of food.	URINE.				Remarks
				Vol.	Sp. gr.	Nitro- gen.	Indigo.	
Dec. 5	kilos 10.5	grams { Cracker-dust 150 }	grams	c.c. 390	1.016	grams 2.70	mgms. 9.2	Defæca- tion.
" 6	10.5	{ Lard . . 75 }	2.50	455	1.009	2.40	0.9	Defæca- tion.
" 7	10.5	{ Water . . 200 }		305	1.011	2.54	0.9	Defæca- tion.
GELATINE PERIOD.								
" 8	10.3	{ Cracker-dust 60 }	10.14	285	1.031	9.90	1.86	No defæ- cation.
" 9	10.3	{ Gelatine . 66 }		235	1.035	9.78	1.46	Defæca- tion.
" 10	10.2	{ Lard . . . 50 }		220	1.038	10.01	2.44	Defæca- tion.
" 11	10.2	{ Water . . 250 }		285	1.038	9.45	2.60	No defæ- cation.
MEAT PERIOD.								
" 12	10.3	{ Cracker-dust 60 }	10.14	180	1.030	5.53	23.68	Defæca- tion.
" 13	10.5	{ Meat . . . 260 }		210	1.028	6.39	13.24	Defæca- tion.
" 14	10.7	{ Lard . . . 50 }						
		{ Water . . 150 }		235	1.030	6.93	18.12	No defæ- cation.

From the preceding tables it is seen that when gelatine is fed to a dog as the chief nitrogenous constituent of the diet, the urinary indican is greatly decreased, or if the indican is decreased by feeding the animal on a diet poor in nitrogen, the subsequent administration of gelatine does not materially increase the output of indican. The absence of kynurenic acid from the urine of the dog during gelatine feeding, as shown by Mendel and Jackson,¹ is of interest in this connection in view of the intimate relationship which Ellinger has lately established between that quinoline derivative and tryptophan.

¹ MENDEL and JACKSON: This journal, 1898-99, ii, p. 1.

EXPERIMENT 4.

MEAT PERIOD.								
Date, 1904.	Weight.	FOOD.	Nitro- gen.	URINE.				Remarks.
				Vol.	Sp. gr.	Nitro- gen.	Indigo.	
Jan. 11	kilos 9.2	grams { Cracker-dust 60	grams	c.c. 116	1.056	grams 6.66	mgms. 34.86	No defæca- tion.
" 12	9.4	{ Meat . . . 260	11.44	215	1.050	10.60	21.94	Defæcation.
" 13	9.5	{ Lard . . . 50		290	1.030	9.44	30.04	No defæca- tion.
" 14	9.6	{ Water . . . 140		275	1.032	8.62	35.92	No defæca- tion.
CRACKER-DUST PERIOD.								
" 15	9.6	{	2.63	120	1.048	2.75	17.50	No defæca- tion.
" 16	9.4	Cracker-dust 150		urine of this day lost.				
" 17	9.3	{ Lard . . . 75		325	1.010	3.60	14.92	No defæca- tion.
" 18	9.2	{ Water . . . 200		250	1.015	3.12	13.02	No defæca- tion.
" 19	9.2	{		175	1.018	2.94	11.20	No defæca- tion.
GELATINE PERIOD.								
" 20	9.2	{ Cracker-dust 50	11.44	240	1.032	9.36	13.02	Defæcation.
" 21	9.2	{ Gelatine . . 74		200	1.036	9.36	13.98	Defæcation.
" 22	9.2	{ Lard . . . 25		100	1.030	2.85	13.98	No defæca- tion.
" 23	9.2	{ Water . . . 250		144	1.022	2.40	14.62	No defæca- tion.
" 24	9.3	{		50	1.038	1.62	0.70	Defæcation.
ZEIN PERIOD.								
" 25	9.3	{ Cracker-dust 150	12.92	200	1.015	3.35	0.82	Defæcation.
" 26	9.3	{ Zein . . . 90		350	1.013	4.56	12.20	No defæca- tion.
" 27	9.3	{ Lard . . . 25		225	1.016	4.14	19.60	No defæca- tion.
		{ Water . . . 250						
MEAT PERIOD.								
" 28	9.3	Diet as in meat period above		185	1.035	7.92	43.40	Defæcation.

On the Origin and Precursors of Urinary Indican. 183

Osborne and Harris¹ have shown that certain proteids of the vegetable kingdom give only a very faint reaction with the Adamkiewicz test. Zein is such a substance, and a single experiment is given on the preceding page to show the influence of zein-feeding upon the excretion of indican.

The daily composition of the urine of this animal shows very wide variations, which are perhaps referable to irregularities in the periods of defæcation, and to variations in the extent of absorption of the food-constituents. Yet even under these conditions the yield of indican is relatively low on the gelatine diet. During the zein period an examination of the fæces showed a very poor utilization of this material. The single experiment will not permit of any broad generalization further than to point out that even under these conditions favorable for putrefactive changes the indican output was far lower than on the meat diet.

These experiments show that not only the quantity of the nitrogenous constituents of the diet is of influence, but the *quality* of these substances plays an important rôle in their bearing upon the excretion of indican. The fact that gelatine will reduce the quantity of indican excreted — that is, causes a diminished formation of putrefactive products — is not alone of theoretical interest, but may prove of clinical importance in the treatment of certain conditions of the intestine where it is desirable to avoid the products of putrefaction.

¹ OSBORNE and HARRIS: *Journal of the American Chemical Society*, 1903, xxv, p. 853.

ETHER-LAKING: A CONTRIBUTION TO THE STUDY
OF LAKING AGENTS THAT DISSOLVE
LECITHIN AND CHOLESTERIN.

By S. PESKIND.

AMONG hæmolytic agents a certain group of substances may be distinguished, the members of which have one characteristic in common; *i.e.*, they all possess the power of dissolving cholesterolin and lecithin. To this group belong, for example, ether, ethyl-alcohol, amyl-alcohol, sodium taurocholate, sodium glycocholate, and sapotoxin. The fact that sapotoxin is an actual solvent of cholesterolin and lecithin was not known to me at the time of the research, but the fact was inferred from the great similarity between the process of ether-laking and that of sapotoxin laking. A recent work by Kobert,¹ which has just come to hand, seems to establish this fact beyond doubt, so that sapotoxin must take a prominent place among the lakers, which are solvents of cholesterolin and lecithin.

Now, it is one thing to know that the substances mentioned are solvents of cholesterolin and lecithin, but it is quite another matter to show that the laking of the blood-corpuscles is actually due to the solution of these substances and their extraction from the corpuscles. Other explanations, possessing a great deal of probability, could be advanced to account for the hæmolysis produced by ether, sapotoxin, and similar agents.

The "Solution Hypothesis," stating that laking by such substances as ether is due to the mechanical solution and extraction from the corpuscles of cholesterolin and lecithin, seems the most reasonable, however, and is the one commonly held. Starting out with this hypothesis as a working theory, a number of experiments were instituted, and the facts thus obtained were applied for and against this theory. As far as my experiments have gone, the evidence is strongly in favor of the "Solution Hypothesis."

¹ R. KOBERT: Beiträge zur Kenntniss der Saponinsubstanzen, 1904, pp. 48-49.

Before the subject-matter is entered into, a brief description of the structure of a mammalian red blood-corpuscle, as it is assumed in this paper, will be given. For reasons which have been given elsewhere, a red corpuscle is considered as possessing an envelope, stroma, and cell-contents.¹ The envelope is hæmoglobin-free, and is not a differentiated membrane, but an integral part of the stroma, which is condensed at the periphery of the corpuscle to form an enveloping layer. This view would make the envelope and stroma of the same physical and chemical structure. The stroma is a network in whose meshes are contained the fluid or semi-fluid contents of the corpuscle, consisting of hæmoglobin and electrolytes. The envelope, as well as the stroma, consists of nucleoproteid, cholesterin, lecithin, and mineral constituents. It possesses a selective permeability.

With this conception of a blood-corpuscle in mind, what process would we expect to occur when the corpuscle is exposed to the action of ether or any other solvent of cholesterin and lecithin? The first thought would be that the solvent extracts the cholesterin and lecithin from the envelope, and thus increases the permeability of the latter. Ions and water would then quickly enter the corpuscle, causing swelling of the latter, followed by laking. And we find, in fact, that ether does produce swelling of the corpuscles before it lakes them. Moreover, an attempt made to determine whether actual solution of cholesterin and lecithin occurred when just sufficient ether was added to cause laking yielded a positive result, from which it appeared that a small but definite proportion of cholesterin and lecithin was removed from the corpuscles during laking by ether. Other experiments will be cited in support of the view that laking is actually due to the solution of these substances from the envelope of the corpuscle.

What is the condition of the cholesterin and lecithin in the corpuscle? Are these substances diffused throughout the stroma in a condition similar to that of the particles of fat in an emulsion, or are they bound to some constituent of the corpuscle by weak chemical ties, comparable, for instance, to the chemical force by which oxygen is held in oxyhæmoglobin? The facts brought out in this paper favor the view that these substances are in an emulsiform condition, and are capable of mechanical solution.

Assuming the cholesterin and lecithin of the corpuscle to be in a physical state, and not in chemical combination, we might, for the purpose of investigation, ignore the rest of the corpuscle and con-

¹ PESKIND: American journal of the medical sciences, 1904, p. 1011.

ceive a given volume of blood merely as a suspension of cholesterin and lecithin. If ether be added to this suspension, then these substances will be dissolved after a certain amount of ether has been added. What relation does this amount of ether bear to the quantity of ether necessary to lake the same volume of blood? It is evident that if ether-laking be due to such a solution of cholesterin and lecithin, some simple quantitative relation should be demonstrable between the above-mentioned quantities of ether.

To bring out such a relation, if it exists, the answers to the following questions were sought experimentally:

1. How much ether is necessary to lake a given volume (5 c.c.) of whole blood?

5 c.c. was employed throughout the research, as it was found the most convenient amount of blood to manipulate. The determinations were made in flat narrow test-tubes, having parallel sides and small necks, so that the blood was in a thin layer, making the end reaction easily visible, while the narrow necks prevented excessive loss of ether. The ether was added from a capillary pipette, at one or two minute intervals, never more than 0.1 c.c. at a time, shaking well after each addition. The microscope was used in many of the following experiments to control the end reaction; *i.e.*, the point when laking was complete. Unless specially indicated, it will be understood that dog's blood was used. By saline is meant 0.9 per cent sodium chloride solution.

RESULTS.

The amount of ether necessary to lake 5 c.c. of whole blood was found to be:

0.38 c.c.	0.39 c.c.	0.39 c.c.	0.40 c.c.	0.39 c.c.	0.38 c.c.	Average = 0.39 c.c.
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2. How much ether is necessary to lake 0.5 c.c., 1 c.c., 2 c.c., 3 c.c., etc., of whole blood diluted with saline so as to make a volume of 5 c.c.?

RESULTS.

0.1 c.c. whole blood diluted with saline to 5 c.c.	required 0.45 c.c. ether for laking.
0.3 c.c. whole blood diluted with saline to 5 c.c.	required 0.38 c.c. ether for laking.
0.5 c.c. whole blood diluted with saline to 5 c.c.	required 0.38 c.c. ether for laking.
0.5 c.c. whole blood diluted with saline to 5 c.c.	required 0.39 c.c. ether for laking.
1.0 c.c. whole blood diluted with saline to 5 c.c.	required 0.46 c.c. ether for laking.
1.0 c.c. whole blood diluted with saline to 5 c.c.	required 0.43 c.c. ether for laking.
2.0 c.c. whole blood diluted with saline to 5 c.c.	required 0.41 c.c. ether for laking.
2.5 c.c. whole blood diluted with saline to 5 c.c.	required 0.39 c.c. ether for laking.
5.0 c.c. whole blood diluted with saline to 5 c.c.	required 0.40 c.c. ether for laking.
Average = 0.40 c.c.	

Therefore the amount of ether necessary to lake fractions of 5 c.c. of whole blood, diluted with saline to make a volume of 5 c.c., is about the same for all fractions. The average is 0.40 c.c. ether, which is about the same as for 5 c.c. whole blood.

3. How much ether is necessary to lake the washed corpuscles of 5 c.c. of blood, when they are suspended in sufficient saline to make a volume of 5 c.c.?

RESULTS.

The twice-washed corpuscles of 5 c.c. blood diluted to 5 c.c. with saline required for laking the following amounts of ether:

0.40 c.c.	0.40 c.c.	0.41 c.c.	0.39 c.c.	0.38 c.c.
Average = 0.40 c.c.				

Therefore the amount of ether necessary to lake the washed corpuscles of 5 c.c. blood, suspended in saline to make a volume of 5 c.c., is 0.40 c.c. (average), practically the same as for 5 c.c. whole blood.

4. How much ether is necessary to lake the washed corpuscles of 0.5 c.c., 1 c.c., 2 c.c., 3 c.c., etc., of blood when suspended in sufficient saline to make a volume of 5 c.c.?

RESULTS.

The twice-washed corpuscles

of 2.5 c.c. blood suspended in saline to a volume of 5 c.c. required 0.42 c.c. ether.
of 2.5 c.c. blood suspended in saline to a volume of 5 c.c. required 0.40 c.c. ether.
of 2.5 c.c. blood suspended in saline to a volume of 5 c.c. required 0.42 c.c. ether.
of 2.5 c.c. blood suspended in saline to a volume of 5 c.c. required 0.42 c.c. ether.
of 2.5 c.c. blood suspended in saline to a volume of 5 c.c. required 0.40 c.c. ether.
of 1.25 c.c. blood suspended in saline to a volume of 5 c.c. required 0.39 c.c. ether.
of 1.25 c.c. blood suspended in saline to a volume of 5 c.c. required 0.38 c.c. ether.
of 1.25 c.c. blood suspended in saline to a volume of 5 c.c. required 0.41 c.c. ether.
of 1.25 c.c. blood suspended in saline to a volume of 5 c.c. required 0.42 c.c. ether.

Average = 0.40 c.c.

Therefore the amount of ether necessary to lake the washed corpuscles of fractions of 5 c.c. blood, suspended in saline to make a volume of 5 c.c., is about the same for all fractions. The average, = 0.40 c.c. ether, is just the same as for the total corpuscles of 5 c.c. blood.

We see from these results that the smallest amount of blood if diluted to an equal volume requires the same amount of ether for laking as a large amount of blood. Moreover, the same holds true for a suspension of washed corpuscles as well as for whole blood, the serum apparently having no influence on the reaction.

This shows that no laking occurs until a certain per cent of ether is present in solution. All corpuscles are laked simultaneously; *i. e.*, practically so. This fact may also be demonstrated microscopically as well as by centrifugalization.

We see also that the amount of ether needed to lake blood depends not on the number of corpuscles, but entirely on the volume of liquid in which the corpuscles are suspended.

Having determined these points, the next step consisted in extracting the washed corpuscles of 5 c.c. blood with ether. The ether extract consisting of cholesterin and lecithin and perhaps other substances was suspended in 5 c.c. saline and then ether added cautiously until the cholesterin and lecithin was dissolved, leaving a clear solution. The amount of ether used was noted.

Method.—The twice-washed corpuscles of 5 c.c. blood were spread out on porcelain and allowed to dry in the air. This took only a few hours. The dried corpuscles were then transferred to a distilling flask having a ground-glass stopper so that no rubber was present to be dissolved by the ether. Then ether (Squibbs), which had been distilled to remove impurities, was poured over the corpuscles and the flask placed on a water-bath. Care was taken to have the temperature of the water below 75° , the decomposing point of lecithin. In about one-half of the experiments the entire process was carried out in the cold. The corpuscles were thus extracted three successive times. Then the ether containing the extracts was filtered, concentrated to a small volume, and allowed to stand in a small beaker or conical graduate until all the ether had evaporated. A little saline was then added and with the finger the cholesterin and lecithin was removed from the beaker walls and suspended in the saline. This was repeated several times until all the extract had been transferred from the beaker into a flattened test-tube with a narrow neck and then sufficient saline was added to make the total volume of the suspension equal to 5 c.c. Ether was then added slowly in portions not greater than 1 c.c., and the test-tube thoroughly shaken after each addition. This was repeated until the cholesterin and lecithin were completely dissolved and a clear solution obtained.

In some experiments a little sapotoxin solution was added to the suspension of cholesterin and lecithin in order to emulsify these substances and make the particles finer, so as to be more easily dissolved by the ether. But this did not prove very successful, inasmuch as the sapotoxin caused a certain amount of opalescence and turbidity (perhaps some of it was precipitated by the ether) so that the end reaction was not sharp.

Again, in other experiments the cholesterin and lecithin were suspended

in serum from the same dog as that from which the blood was taken. The end reaction in these cases was very sharp, and the results were the same as when saline was used as a medium of suspension.

5. **Results.**—The ether extract of the corpuscles of 5 c.c. dog's blood suspended in 5 c.c. saline required for solution the following amounts of ether:¹

0.46 c.c. 0.44 c.c. 0.45 c.c. 0.51 c.c. 0.49 c.c. Average = 0.47 c.c.

The ether extract of the corpuscles of 5 c.c. dog's blood suspended in 5 c.c. serum from the same dog required for solution the following amounts of ether:—

0.52 c.c. 0.48 c.c. 0.48 c.c. 0.48 c.c. Average = 0.49 c.c.

Therefore the amount of ether required to dissolve the cholesterin and lecithin extracted from the corpuscles of 5 c.c. blood, suspended in 5 c.c. saline, is 0.47 c.c. (average), suspended in serum 0.49 c.c. (average). Average of both methods of suspension, 0.48 c.c.

6. The extract of the corpuscles of 5 c.c. blood was divided into fractions and these fractions were suspended in 5 c.c. saline. The amount of ether necessary to dissolve these fractions was noted.

RESULTS.²

The ether-extract of the corpuscles

- of 2.5 c.c. blood suspended in 5 c.c. saline required for solution 0.49 c.c. ether.
- of 2.5 c.c. blood suspended in 5 c.c. saline required for solution 0.48 c.c. ether.
- of 2.0 c.c. blood suspended in 5 c.c. saline required for solution 0.46 c.c. ether.
- of 2.5 c.c. blood suspended in 4 c.c. saline + 1 c.c. of 1 % sapotoxin solution required for solution 0.43 c.c. ether.

Average = 0.465 c.c.

Therefore the amounts of ether required to dissolve the fractions of the cholesterin and lecithin extracted from the corpuscles of 5 c.c. blood and suspended in 5 c.c. saline is about the same as for the total extract from 5 c.c. Average = 0.465 c.c.

From these experiments (5 and 6), we see that it takes the same amount of ether to dissolve a fraction of the cholesterin and lecithin extracted from the corpuscles of 5 c.c. blood and suspended in 5 c.c. saline as it does to dissolve the total quantity of the extract.

¹ The higher values, 0.51 c.c. and 0.49 c.c., are undoubtedly the correct ones. They were obtained in cool weather without the addition of sapotoxin. This made the end reaction very sharp. In warm weather the cholesterin and lecithin forms a smeary flocculent precipitate after a little ether is added, so that the end reaction is not quite so easily judged.

² The higher values, 0.49 c.c. and 0.48 c.c., are the better ones, for reasons given in preceding footnote.

Therefore there must be a certain per cent of ether in solution in order to dissolve the cholesterol and lecithin contained in the corpuscles of 5 c.c. blood and suspended in 5 c.c. saline.

We have previously determined that a certain per cent of ether must be in solution in order that blood-corpuscles may be laked. Putting these two findings together, it seems reasonable to conclude that the laking of the corpuscles depends on the solution of the cholesterol and lecithin contained in them, since both processes follow similar laws.

Since the smallest amount of cholesterol and lecithin extract takes as much ether for solution as the larger amounts, provided the volume is constant, and since we assume that ether-laking is due to extraction of some of the cholesterol and lecithin contained in the corpuscles, we would expect that the amount of ether necessary to lake 5 c.c. of blood would be exactly the same as the amount of ether necessary to dissolve the cholesterol and lecithin extracted from 5 c.c. blood and suspended in 5 c.c. saline. But the amounts differ. We find that it takes 0.39 c.c. ether to effect laking of 5 c.c. blood, whereas 0.48 c.c. ether are required to dissolve the cholesterol and lecithin extracted from the corpuscles of 5 c.c. blood and suspended in 5 c.c. saline. How can we reconcile our assumption with these facts?

A little consideration will show that there is no real discrepancy between the actual findings and those demanded by theory.

In 5 c.c. of blood we have but 40 per cent corpuscles and 60 per cent serum.¹ When we add the ether to the blood, it does not penetrate immediately, so that it is really dissolved in only 60 per cent of 5 c.c., or 3 c.c. of solution. The ether remains outside of the corpuscles until the cholesterol and lecithin have been dissolved out from the envelope, at which point the corpuscles have reached the condition necessary for laking.

Therefore the two experiments to determine the amount of ether necessary to lake blood and the amount of ether required to dissolve the cholesterol and lecithin of the corpuscles were performed under unequal conditions as regards volume; the volume of the solution in which the ether was dissolved in the former case being really only 3 c.c., while in the latter case it is 5 c.c., — a difference in volume of 2 c.c.

¹ HAMBURGER: *Jonenlehre*, 1902, p. 507.

Of course it would take less ether to dissolve the cholesterin and lecithin in the former case than in the latter. The proper way to have made the experiment would have been to have suspended the extract of cholesterin and lecithin in 3 c.c. saline instead of 5 c.c.

If 5 c.c. blood is suspended in sufficient saline to make 7 c.c., it requires 0.49 c.c. ether to lake. Now, 5 c.c. blood contains 2 c.c. corpuscles. Therefore in this suspension the actual volume of liquid is 7 c.c. minus 2 c.c. = 5 c.c. Therefore it takes 0.49 c.c. ether to lake a blood suspension in which the actual volume of liquid (or solution) is 5 c.c. This corresponds exactly with the amount of ether required to dissolve the lecithin and cholesterin contained in the corpuscles of 5 c.c. blood and suspended in 5 c.c. saline.

The explanation here given, based on the assumption that the ether does not penetrate the corpuscles immediately, seems to be borne out by the facts and data given. Moreover, if this explanation be correct, we would expect that 5 c.c. of whole blood would require less ether for laking than would 1 c.c. of blood diluted with saline to make a volume of 5 c.c., since, on account of the greater number of corpuscles, the actual volume of liquid is less in the first case than in the second. Therefore less ether ought to be required in the former case than in the latter.

Indications that this is actually so are to be found in the data given above (tables). Whereas, in the table given for the amount of ether which is needed to lake 5 c.c. of blood, no figures higher than 0.41 c.c. are to be found, we find such figures as 0.46 c.c., 0.45 c.c., 0.43 c.c., for lesser quantities of blood diluted to 5 c.c. with saline.

It is rather difficult in working with such small amounts of ether to get results uniformly accurate to the hundredth of a cubic centimetre, since evaporation and other factors hinder to a slight extent. If we work with larger volumes of blood suspension, such as 7 c.c., the error is less, and we can show that it really does require less ether to lake the blood-suspension having the greatest number of corpuscles.

EXPERIMENT.

5 c.c. of whole blood diluted with saline to 7 c.c. required 0.49 c.c. ether to lake.

2 c.c. blood diluted to 7 c.c. required 0.57 c.c. ether to lake.

2 c.c. blood diluted to 7 c.c. required 0.53 c.c. ether to lake.

Average of last two results = 0.55 c.c.

These experiments show that it requires less ether to lake the suspension having the greatest number of corpuscles, the total volume being the same.

$0.55 \text{ c.c.} - 0.49 \text{ c.c.} = 0.06 \text{ c.c.}$ = Difference between the amount of ether required to lake 2 c.c. and 5 c.c. of whole blood respectively, when suspended in sufficient saline to make 7 c.c.

Therefore a difference of 3 c.c. blood is represented by a difference of 0.06 c.c. in the amount of ether required to cause laking.

But 3 c.c. blood contains 40 per cent or 1.2 c.c. of serum.

So that, in the case where 2 c.c. blood was suspended to make 7 c.c., there was 1.2 c.c. more liquid present than in the case where 5 c.c. blood was diluted to 7 c.c.

A difference of 1.2 c.c., therefore, in the actual volume of liquid in the blood suspension requires 0.06 c.c. more ether to effect laking; or an increase of 1 c.c. in volume requires an increment of 0.05 c.c. in the amount of ether required.

In 5 c.c. blood the actual volume is 3 c.c., and the amount of ether required to effect laking is 0.39 c.c. (See table.)

To find how much ether is required to lake 5 c.c. blood + 2 c.c. saline (= 7 c.c.), we add $2 \times 0.05 \text{ cc.} = 0.10 \text{ c.c.}$ to 0.39 c.c. = 0.49 c.c. = amount of ether necessary to lake 5 c.c. blood diluted to 7 c.c., as found by theoretical calculation. The actual quantity of ether found necessary by experiment was also 0.49 c.c.

All these facts seem to be in harmony with the assumption that the per cent of ether required to lake blood-corpuscles is really the same as the per cent of ether required to dissolve the cholesterin and lecithin extracted from the corpuscles and suspended in saline. This would justify the conclusion that when ether is added to a blood suspension, the laking produced is due to the extraction of the cholesterin and lecithin from the corpuscles.

So far we have considered the ether-extract in the aggregate. Let us now attempt to differentiate the various constituents of the ether-extract, to determine, if possible, whether one or all of these substances are concerned in ether-laking.

Experiment. — The amount of cholesterin and lecithin present in the corpuscles of 5 c.c. blood having been determined, a similar quantity of pure cholesterin (Merck's), was suspended in 5 c.c. saline. Various fractions of this amount of cholesterin were also suspended in 5 c.c. saline.

It required the same quantity of ether to dissolve the larger quantity of cholesterin as it did to dissolve any of the fractions. Average amount of ether required was 0.51 c.c.

Results in detail. — A suspension of cholesterin in 0.9 per cent sodium-chloride solution was made in the proportion of 10 mg. of the former to

5 c.c. of the latter. The suspension was boiled and cooled to convert the cholesterin into fine globules, so as to be easily soluble in ether.

5.0 c.c. of this cholesterin suspension required 0.54 c.c. ether for solution.

2.5 c.c. of this cholesterin suspension diluted with saline to 5 c.c. required 0.5 c.c. ether for solution.

2.0 c.c. of this cholesterin suspension diluted with saline to 5 c.c. required 0.5 c.c. ether for solution.

1.0 c.c. of this cholesterin suspension diluted with saline to 5 c.c. required 0.5 c.c. ether for solution.

The end reaction was very sharp.

From these experiments we learn that the solution of a cholesterin suspension by ether follows the same laws as the solution of the ether-extract of the corpuscles, and this in turn is subject to the same laws as the laking of blood-corpuscles by ether.

We may assume, therefore, that the extraction of cholesterin from blood-corpuscles by ether produces laking of the former.

Is it essential that cholesterin be dissolved from the corpuscles by ether in order that laking should occur? Can ether produce laking without the extraction of cholesterin? Apparently it can, as would appear from the subjoined experiments. If, as we have reason to suppose, the cholesterin in the blood-corpuscles is the same as the cholesterin obtained from other sources, we should expect that ether saturated with cholesterin would not remove any cholesterin from blood-corpuscles. Moreover, if the addition of this cholesterin-saturated-ether should cause laking, we would be quite safe in assuming that the laking was not due to the extraction of any cholesterin contained in the corpuscles.

These questions were investigated by aid of the following special solutions:

0.9 per cent sodium chloride solution was saturated with ether. This solution we shall designate as ether-saline. Some of this ether-saline was thoroughly saturated with cholesterin (Merck's).¹ This solution we shall designate cholesterin-ether-saline.

¹ To insure a complete and perfect saturation of the ether-saline with cholesterin the following procedure was employed. An ether wash-bottle (all the parts of which are of glass, and which has one tube extending to the bottom, while the other enters at the top) was partly filled with saline. Enough ether was then added to saturate the saline and to form a half-inch surface layer. Then a considerable quantity of cholesterin was added to this mixture and shaken thoroughly until all the cholesterin was dissolved. Of course most of the cholesterin was taken up by

0.9 per cent sodium chloride solution was saturated with chloroform. This we shall call chloroform-saline.

Some of the chloroform-saline was saturated with cholesterin. This we shall call cholesterin-chloroform-saline.

Some pure ether was saturated with cholesterin. This we shall designate as cholesterin-saturated-ether.

Some chloroform-saline was saturated with lecithin (Merck's). This we shall call lecithin-chloroform-saline.

EXPERIMENTS.¹

1. To portions of 5 c.c. cholesterin-ether-saline added 0.1 c.c. blood.
Complete laking took place at the end of the following periods :
5 minutes. 6 minutes. 5 minutes. Average = 5.3 minutes.
2. To portions of 5 c.c. ether-saline added 0.1 c.c. blood.
Complete laking occurred at the end of the following periods :
5.5 minutes. 4.5 minutes. 4.5 minutes. 5.5 minutes. Average = 5 minutes.
3. To portions of 5 c.c. chloroform-saline added 0.1 c.c. blood.
Complete laking occurred after
18 minutes. 16 minutes. 18 minutes. Average = 17.3 minutes.
4. To portions of 5 c.c. chloroform-cholesterin-saline added 0.1 c.c. blood.
Complete laking occurred after
14 minutes. 18.5 minutes. Average = 16.25 minutes.
5. To portions of 5 c.c. chloroform-lecithin-saline added 0.1 c.c. blood.
Complete laking after
3.5 minutes. 3.5 minutes. Average = 3.5 minutes.

the surface layer of ether. The ether was allowed to evaporate slowly during a period of several days, the wash-bottle being shaken at frequent intervals to insure a thorough saturation of the ether-saline with the cholesterin. When sufficient ether had evaporated, the cholesterin began to separate out from the layer of ether on top of the saline. No amount of shaking would dissolve the cholesterin which had separated out, either in the surface layer of ether or in the solution of ether-saline underneath. We had therefore a top layer of ether that was perfectly saturated with cholesterin, and a saline solution underneath that was completely saturated with ether and cholesterin. One tube of the wash-bottle extended to the bottom of the lower layer, and, whenever some of this cholesterin-ether-saline was desired for the experiment, it was forced out through this tube.

¹ Dog's blood was used in these experiments.

(The lecithin was probably somewhat decomposed, and the presence of glycerophosphoric acid probably accounts for the shortening of the laking period from 17 minutes to 3.5 minutes).¹

6. To 5 c.c. of ether-saline + 0.1 c.c. blood added saline to make 7 c.c. Laked after 34 minutes.
To 5 c.c. of ether-saline + 1 c.c. blood added saline to make 7 c.c. Laked after 34 minutes.
To 5 c.c. of ether-saline + 2 c.c. blood added saline to make 7 c.c. Laked after 35 minutes.
- 6a. To 5 c.c. of ether-cholesterin-saline + 2 c.c. blood added saline to make 7 c.c. Laked after 30 minutes.

EXPERIMENTS WITH SHEEP'S BLOOD.

7. To 10 c.c. ether-saline added 1 c.c. blood. Laked completely in 3 minutes.
8. To 5 c.c. ether-saline added 2 c.c. blood. Laked completely in 26 minutes.
9. To 5 c.c. cholesterin-ether-saline added 0.5 c.c. blood. Laked completely in 30 minutes.
10. 5 c.c. blood required 0.37 c.c. ether to lake.
11. 5 c.c. blood + 0.33 c.c. cholesterin-saturated-ether showed no laking after 5 minutes. Then 0.04 c.c. more of cholesterin-saturated-ether was added; laking took place instantly.
Therefore 5 c.c. blood required 0.33 c.c. + 0.04 c.c. = 0.37 c.c. of cholesterin-saturated-ether.
12. 5 c.c. blood + 0.4 c.c. cholesterin-saturated-ether. Laked within 2 minutes.

Comparing Experiments 1 and 2, it will be observed that cholesterin-ether-saline lakes blood in practically the same time as ether-saline, the conditions being the same.

Experiments 3 and 4 show that chloroform-cholesterin-saline lakes blood in practically the same time as chloroform-saline, the conditions being equal.

Experiments 10 and 12 show that it takes the same amount of cholesterin-saturated-ether to lake blood as it does of pure ether, the conditions being the same.

All these experiments show that the previous saturation of ether with cholesterin does not in the least influence its laking power.

¹ This explanation may not be correct, however, since it has been shown by KYES and SACHS that pure lecithin has a weak hæmolytic action. ROBERT (Saponinsubstanzen, p. 50) corroborates this observation, and states that he has found lecithin from eggs to have a hæmolytic action.

Ether saturated with cholesterin lakes blood just as though no cholesterin were dissolved in it. The same holds true for chloroform.

Another deduction to be made is that although the removal of cholesterin from the corpuscles was prevented by previous saturation of the ether with cholesterin, yet laking occurred as usual, the usual per cent of ether and the usual time being required.

By analogy with previous cholesterin experiments (see page 192), we may assume that the laking in these cases was due to the extraction of lecithin from the corpuscles (provided, of course, that the removal of some other ether-soluble substance, the existence of which has been suspected by some investigators, was not the cause of the laking).

From Experiment 6 it will be seen that solutions containing the smallest quantity of blood laked in the same time as the solutions containing the larger amounts. Since all of these suspensions were of the same volume, and therefore contained the same quantities of ether, and since the smallest number of corpuscles required as long a time for laking as the larger quantities of blood, it becomes evident that ether must first produce a definite alteration in the corpuscles before they are ready to lake. This change, which requires a definite time for ether to bring about, is visible under the microscope as a swelling of the corpuscles. We shall show later on that some chemical change is also demonstrable.

CHEMISTRY OF ETHER-LAKING.

In order to prove the "Solution Hypothesis" we must demonstrate that ether actually dissolves cholesterin and lecithin from the corpuscles during laking. This involves a quantitative determination of the amounts of cholesterin and lecithin in blood-corpuscles before and after laking by ether. While such a determination involves several difficulties, a fairly accurate estimation may be made through a method developed in this research.

The problem requires that a given amount of blood be laked by an exactly sufficient quantity of ether; as soon as laking has occurred, the stromata must be quickly removed by centrifugalization before the ether evaporates sufficiently to allow any of the cholesterin and lecithin which it dissolved from the corpuscles to separate out from solution; then the stromata must be washed, again centrifugalized, dried, and extracted by ether.

To answer these requirements, a method of precipitation must be employed. Heretofore sodium bisulphate has been used to precipitate stromata, but a quantitative precipitation by this agent cannot be accomplished. In a previous article,¹ a method of agglutinating and precipitating blood-corpuscles and their stromata by means of acids and acid salts has been described. If exactly sufficient amounts of these reagents are added, the corpuscles and stromata are agglutinated into clumps and precipitated, but after being washed with saline, the agglutinated masses tend to break up, and the corpuscles and stromata resume a more or less normal suspension. To prevent this, more reagent must be added during washing. While this is feasible with corpuscles, it is not practical to do so with stromata, because a slight excess of most acids and acid-salts — such as hydrochloric acid or ferric chloride — disintegrates and even dissolves the stromata. Moreover, most acid-salts precipitate hæmoglobin more or less from laked blood, and this prevents us from judging when all the stromata have been precipitated.

A reagent was therefore sought that was only slightly soluble in water or saline, so that any considerable excess would at no time be present in solution, — one that would precipitate stromata quantitatively from laked blood without injuring them, and that would not precipitate hæmoglobin from the laked blood.

All these requirements were found to be very satisfactorily answered by iron hypophosphite. This is an acid salt that is but slightly soluble in saline or water. It precipitates corpuscles and stromata, while it exerts a minimum of destructive action on them. It may be added in bulk to the blood-suspension, or laked blood, and will precipitate quantitatively the corpuscles or stromata that are present, the excess of iron salt settling to the bottom. When the precipitate is centrifugalized and washed, the excess of iron hypophosphite that is present will prevent the corpuscles or stromata from regaining their normal suspension.

EXPERIMENTS.

A and D. — To the twice-washed corpuscles of 2.5 c.c. blood added 1 c.c. of a suspension of 2 grams iron hypophosphite in 10 c.c. saline, diluted with saline to 5 c.c. and shook. The corpuscles were immediately precipitated. Then added 0.42 c.c. ether and shook thoroughly. The corpuscles were completely laked, leaving their stromata in agglutinated clumps.

¹ PESKIND: This journal, 1903, viii, p. 404.

The corpuscles of 10 c.c. blood were thus prepared simultaneously in 4 tubes, and after the corpuscles were laked the mixtures were transferred to centrifuge tubes and quickly centrifugalized. The sediment, consisting of stromata and excess of iron hypophosphite, was washed twice and centrifugalized after each washing. The washed sediment was then placed on porcelain and allowed to dry at room temperature. The dry sediment was then ground to a fine powder, placed in a beaker, and extracted with ether three times in the cold and once at boiling temperature. The ether was allowed to evaporate and the extract weighed.

B and C. — The twice-washed corpuscles of 2.5 c.c. blood — diluted to 5 c.c. with saline — were laked with 0.42 c.c. ether. To the laked blood added 1 c.c. of the hypophosphite of iron suspension and shook. The stromata were precipitated. The corpuscles of 10 c.c. blood were thus prepared and then the mixture was centrifugalized. The rest of the procedure detailed under A and D followed.

E. — The twice-washed corpuscles of 10 c.c. blood were dried in the air, on porcelain. They were then extracted with ether three times in the cold and twice with heat. After evaporation of the ether the extracts were weighed.

F. — The same as E, only the dried corpuscles were extracted three times with boiling ether, and not all with cold ether.

The ether extract obtained from the stromata had a yellow color, such as is seen in old lecithin solutions. This is probably due to a slight decomposition of the lecithin produced by the excess of iron hypophosphite present in the sediment of stromata. Such a decomposition would very likely be prevented by mixing the washed sediment (consisting of stromata and iron hypophosphite) with calcium carbonate before drying. This yellow substance could not be re-dissolved in cold ether. It is well known, however, that when lecithin is deposited from an ethereal solution (containing water) it becomes quite insoluble in ether, so that in all probability the yellow substance was lecithin. The amount of insoluble yellow substance was very small and about the same in all experiments. If we include this in the whole extract as lecithin, then we find very little difference between the amounts of cholesterin and lecithin in the corpuscles before and after laking with ether. Roughly, about 10 per cent less of these substances were found in the stromata after laking by ether than was present in the intact corpuscles. By working with large quantities of blood, the exact amount of cholesterin and lecithin removed during laking could, I believe, be determined quite accurately, while the proportions of the different constituents removed

might perhaps also be determined by quantitative analysis. These determinations will be attempted in a succeeding research.

RESULTS.

E. 0.0087 gm. = amount of cholesterin and lecithin in the corpuscles of 10 c.c. blood.

F. 0.0080 gm. = amount of cholesterin and lecithin in the corpuscles of 10 c.c. blood.

Average = 0.00835 gm.

The stromata of the washed corpuscles of 10 c.c. blood laked by ether were found to contain —

A. B. D.

0.0071 gm. 0.006 gm. 0.0061 gm. of cholesterin and lecithin, not including the yellow substance.

0.0078 gm. 0.008 gm. 0.0072 gm. of cholesterin and lecithin, including the yellow substance as lecithin.

Average = 0.0064 gm. cholesterin and lecithin, not including yellow substance.

Average = 0.00767 gm. cholesterin and lecithin, including yellow substance.

0.0083 gm. = Average amount of cholesterin and lecithin in the corpuscles of 10 c.c. blood.

0.0064 gm. = Average amount of cholesterin and lecithin in the stromata, not including yellow substance.

0.0019 gm. = Differences in the amount of cholesterin and lecithin in the corpuscles before and after laking if the yellow substance be not included as part of the ether extract.

0.00835 gm.

0.00767 = Average amount of cholesterin and lecithin in the stromata, including the yellow substance as lecithin.

0.0007 gm. = Difference in amount of cholesterin and lecithin before and after laking if the yellow substances be included as lecithin.

These experiments show that a comparatively small proportion of cholesterin and lecithin is dissolved out of the corpuscles during ether-laking. Moreover, we know that in order to produce laking, ether must bring about a certain change in the corpuscles, this change in all probability being effected by the ether before it penetrates to the interior of the corpuscles. (See pages 190-191.)

The deduction naturally follows that the primary effect of the ether is to dissolve out the cholesterin and lecithin from the surface-layer of the corpuscle or envelope. After these substances have been dissolved from a layer of definite thickness, a sufficient increase in permeability of the envelope is brought about to admit of the rapid entrance of ions and water, giving rise to laking.

If, by using large amounts of blood and otherwise perfecting the method of determining the cholesterin and lecithin in stromata, we shall be able to make a very accurate quantitative estimation of these substances, it seems quite possible that, knowing the diameter of the corpuscles and the relative amounts of nucleoproteid, cholesterin,

and lecithin in the corpuscles and stromata, we should be able to calculate approximately the thickness of the envelope.

DISCUSSION.

While the experimental results cited in this paper are favorable to the "Solution Hypothesis" of ether-laking, yet the evidence is not complete, and certain other experiments, to be mentioned below, will have to be performed before a final decision as to the correctness of this theory can be reached.

There is no doubt that ether produces laking of a blood-corpuscle through an action which it exerts on some ether-soluble constituent present in the envelope. But what is this action? Is it simply a solution and extraction of cholesterin and lecithin from the envelope, or does it consist in a modification of the physical properties of these substances without causing their actual solution? The following experiment throws light on this question and will help us in our conception of the processes that occur in ether-laking.

If lecithin, or a mixture of lecithin and cholesterin, is brought into an aqueous solution of ether, a portion of the ether will be removed from the aqueous solution and will be absorbed or dissolved by the lecithin-cholesterin mixture, the proportion of ether taken up in this way being determined by the "coefficient of partition" of ether between the two solvents, water and the lecithin-cholesterin mixture. This "partition coefficient" is the same for all concentrations of the ether.¹

How can we utilize these facts to explain the phenomena of ether-laking?

Granting that the cholesterin and lecithin of blood-corpuscles exist in a physical state and not in chemical combination, we would expect that when ether is added to a blood-suspension, part of the ether would be withdrawn from the aqueous (or rather saline) solution and absorbed by the cholesterin and lecithin in the envelopes of the corpuscles. It is very likely that up to a certain point the cholesterin and lecithin may dissolve ether without undergoing a sufficient physical change to cause any considerable alteration in the osmotic properties of the envelope.² If still more ether be taken up, however, the cholesterin and lecithin become so greatly modified in their

¹ E. OVERTON: Studien über die Narkose, 1901, pp. 57, 69.

² *Ibid.*: p. 178.

physical properties that an increased permeability of the envelope results.

That an increase of permeability, produced in this way without actual solution of the cholesterol and lecithin, is sufficient to produce laking, is not at all improbable, and as an alternative to the "Solution Hypothesis" the theory just outlined will explain most of the facts.¹

We have reasons for believing that the processes just described as possibly occurring in ether-laking actually do take place.

If ether be added cautiously to a blood-suspension having a volume of 5 c.c., the corpuscles undergo but little change until 0.3 c.c. of ether has been added. Then swelling of the corpuscles occurs. This shows that an increase of permeability has been effected by the action of the ether on the envelope of the corpuscles. (No laking takes place until 0.39 c.c. ether has been added.)

If all or part of the cholesterol and lecithin extracted from the corpuscles of 5 c.c. blood be suspended in 5 c.c. saline and ether added carefully, it will be observed that after 0.35 c.c. ether has been added a rather sudden change in the appearance of the suspended cholesterol and lecithin takes place (0.49 c.c. ether being required to dissolve the substances completely). The crystalline and fine granular particles of the suspended cholesterol and lecithin become converted into a flocculent-smeary precipitate which adheres to the side of the test-tube. This change in the physical properties of the cholesterol and lecithin when a certain per cent of ether is present in solution corresponds very well to the physical changes in the corpuscles produced by a similar per cent of ether, and it seems logical to consider such an alteration in the cholesterol and lecithin of the envelope as the primary cause of the increased permeability and swelling of the corpuscles produced by ether.

¹ In this connection I should like to suggest that water-laking may be explained on a similar hypothesis. The commonly accepted theory is that water lakes merely by abstraction of salts from the corpuscles. But a chemical effect is undoubtedly produced by water, since "well-marked agglutination precedes the laking of unfixed blood by water" (G. N. STEWART: *Journal of medical research*, viii, No. 1). A similar agglutination occurs on adding acids and acid salts to blood-corpuscles; these act by modifying the nucleoproteid of the envelopes, so that it becomes sticky. Lecithin also becomes sticky and swells under the influence of water. Therefore the action of water on blood-corpuscles probably consists in modifying the physical properties of both the nucleoproteid and lecithin of the envelopes to such a degree as to produce an increased permeability of the envelopes.

It will be observed that the amount of ether necessary to produce swelling of the corpuscles (0.3 c.c.) is only slightly less than the amount of ether necessary to produce laking (0.39 c.c.); while the amount of ether required to alter the physical properties of the cholesterin (0.35 c.c.) is only a little less than the amount required to produce solution of these substances (0.48 c.c.).

If laking take place at the point when the cholesterin and lecithin of the envelope have absorbed a quantity of ether almost but not quite sufficient to cause their solution, it will be seen that the addition at this stage of only a very slight excess of ether would cause the solution of these substances and consequent extraction from the envelope, making it appear as though the solution of the cholesterin and lecithin were the primary cause of the laking.

This explanation would account for the fact that all the experimental data obtained in this research favor the "Solution Hypothesis." But, after all, it is only a matter of degree, — if laking take place while the cholesterin and lecithin are on the point of solution, or if laking occur after solution of these substances has been effected. The following experiments serve as a means of deciding this question.

Experiments. — When ether is added to blood, the corpuscles swell just before laking occurs. Is this swelling consequent to the removal of cholesterin and lecithin from the envelope, or is it due to the modification of these substances by ether which they have absorbed?

One method of determining this would be to add sufficient ether to a blood-suspension to bring the corpuscles to a stage of great swelling without the production of any laking. This could be called the pre-laking stage of the corpuscles. At this point the corpuscles might be precipitated by an acid salt — preferably the hypophosphite of iron — and the amount of the cholesterin and lecithin in the corpuscles then determined. If no cholesterin and lecithin have been dissolved by the ether, this will be indicated by the results.

Another method of demonstrating which of the two processes is concerned in ether-laking is the following:

Corpuscles swollen by ether so as to be in the prelaking stage — *i. e.*, a stage just short of laking — are placed in a concentrated solution of sodium chloride. Now, if the swelling of the corpuscles is brought about merely through a modification of the cholesterin and lecithin in the envelope, and not through the removal of these substances, then the corpuscles should shrink and resume a normal or subnormal size in the strong saline solution, and after these corpus-

cles are washed entirely free from ether, they should not lake on standing. If these corpuscles be again treated with ether, they should require the same amount and the same time to lake as normal corpuscles.

However, if the swelling of these corpuscles be due to the actual removal or extraction of cholesterin and lecithin from the envelope, then after being washed free from ether the swollen corpuscles should lake in a short time, — either with or without the addition of a small amount of ether.

Again, if ether-laking be due to the solution and extraction of some ether-soluble constituents from the corpuscles, then we would expect to find that ether saturated with the ether-extract of blood-corpuscles would be unable to produce laking. If laking should still occur, however, on treating blood with ether that has been saturated with the ether-extract of blood-corpuscles, then the "Solution Hypothesis" would become quite untenable and we should have to take recourse to the other theory; *i. e.*, the one which assumes that only a modification of the cholesterin and lecithin is necessary to produce laking.

To show that we need not necessarily assume that cholesterin and lecithin must be dissolved from the envelope in order that laking should occur, let us consider the case where blood is laked by cholesterin-saturated-ether or by cholesterin-ether-saline (we have elsewhere (page 196) explained the laking produced by cholesterin-saturated-ether as being due to the extraction of lecithin from the envelope). Assuming, as in all probability is the case, that the cholesterin in the ethereal solution is unable to penetrate into the envelope, while the ether is able to do so, we would expect some of the latter to be taken up by the cholesterin and lecithin of the envelope. As soon as the requisite per cent of ether is in solution, the cholesterin and lecithin become so altered that an increased permeability of the envelope results. The entrance of ions and water might thus be favored to such a degree as to produce laking of the corpuscles. This explanation would account for the fact that *the same per cent* and *the same time* are required to effect laking of blood by cholesterin-saturated-ether as by pure ether.

When the cholesterin-ether-saline is added to blood, the penetration of ether into the envelope of the corpuscles would involve a slight precipitation of the cholesterin outside of the corpuscles. This I have not observed, but the amount of ether entering the corpuscles before laking is probably so small that the precipitation of cholesterin would be hardly appreciable.

It will be seen from the above considerations that a more extended investigation of the subject must be carried out before a definite conclusion can be reached. An attempt will be made in a succeeding research to work out the problems suggested in this paper. The study will be applied not only to ether-laking, but to other solvent-lakers such as chloroform, ethyl-alcohol, amyl-alcohol, sapotoxin, sodium-taurocholate.

Beside the problems already suggested, the following may be mentioned as relevant to the study of ether-laking.

1. Does ether penetrate to the interior of the blood-corpuscles before the lecithin and cholesterin of the envelope is dissolved?

From considerations that have been given in another part of this paper (page 191) it appears highly probable that no such penetration occurs before laking.

2. Is the permeability of the envelope suddenly increased just before laking, *i. e.*, when all the lecithin and cholesterin of the envelope is dissolved out, or does it gradually increase as the solvent action of the ether on the envelope progresses?

This may be determined by electrical conductivity measurements made at various intervals in a blood-suspension which has been treated with ether.

3. After the increased permeability of the corpuscles has been produced through the action of ether, can the swelling of the corpuscles, *i. e.*, the entrance of ions and water, be retarded by increasing the amount of salts in the solution about the corpuscles?

A test experiment made on this point gave the following result:

0.5 c.c. blood + 4.5 c.c. of 1.5 per cent saline required 0.47 c.c. ether to lake.

Control experiment. — 0.5 c.c. blood + 4.5 c.c. of 0.9 per cent saline required 0.40 c.c. ether to lake.

4. The first stage of sapotoxin-laking consists in an increased permeability of the corpuscles. Now, suppose blood-corpuscles are treated with just enough ether to produce a stage when they are just ready to lake, but have not yet done so; *i. e.*, till their permeability has been increased and the corpuscles have all become swelled. If we at this point add sapotoxin, do we still get the initial increase of permeability ordinarily produced by sapotoxin, or do the other stages of sapotoxin action follow? In other words, is the increased permeability produced by sapotoxin of a similar character as that

produced by ether ; *i. e.*, is it due to the modification of, or an actual solution of the cholesterol and lecithin in the envelope? This is a question which may be investigated by electrical conductivity measurements and also by quantitative analysis of the amount of cholesterol and lecithin in corpuscles before and after laking by sapotoxin.

The same problem must be worked out with regard to sodium-taurocholate, which is also a solvent of cholesterol and lecithin.

5. All the laking agents which are solvents of cholesterol and lecithin — *e. g.*, ether, chloroform, alcohol, sapotoxin, sodium-taurocholate — lower the surface tensions of the solutions to which they are added. It would be interesting to determine if any relation exists between the laking power of a substance and the degree to which it is able to depress the surface tension of a solution.

SUMMARY.

The results of the experiments detailed in this paper and the conclusions derived from them may be summed up as follows :

1. For a given volume of any blood-suspension a definite per cent of ether is required to produce laking.

2. The cholesterol and lecithin extracted from blood-corpuscles and suspended in saline requires a definite per cent of ether for solution.

3. The absolute volume of a blood-suspension is the total volume minus the volume occupied by the corpuscles. If we compare the per cent of ether required to lake blood-corpuscles suspended in saline, we find it to be the same as the per cent of ether required for the solution of the cholesterol and lecithin of the corpuscles suspended in the same absolute volume.

4. The conclusion may therefore be drawn that the solution of cholesterol and lecithin from the corpuscles produces laking of the latter, since both processes require the same per cent of ether.

5. Pure cholesterol suspended in a given volume of saline requires for solution the same per cent of ether as is required to lake blood-corpuscles suspended in the same absolute volume.

We may conclude from this that the extraction of cholesterol from the corpuscles produces laking.

6. Ether saturated with cholesterol lakes blood just as though no cholesterol were dissolved in it. The same holds true for chloroform.

Since the extraction of cholesterin from the corpuscles is prevented by previous saturation of the ether with cholesterin, we must ascribe the laking produced in this case to the extraction of lecithin from the corpuscles.

7. Quantitative analysis shows that a small proportion of cholesterin and lecithin is removed from the corpuscles during ether-laking.

8. We have reasons for believing that the cholesterin and lecithin thus removed during laking is extracted from the envelopes of the corpuscles, and that no penetration of the ether into the corpuscles occurs until these substances have been removed from the envelopes.

9. When ether is added to a blood-suspension, some of the ether is absorbed by the cholesterin and lecithin in the envelopes of the corpuscles. Up to a certain point these substances may take up ether and not undergo sufficient change to affect the osmotic properties of the envelope; but as more ether is taken up, the cholesterin and lecithin become so modified in their physical properties that the permeability of the envelope becomes increased. It is conceivable that laking may occur at a point when almost but not quite sufficient ether has been absorbed by the cholesterin and lecithin of the envelope to produce their solution; so that only a very slight excess of ether added at this stage would cause the solution of these substances. This would create the impression that the actual solution of the cholesterin and lecithin was the cause of the laking.

While our findings are in favor of the hypothesis that the cause of ether-laking is the solution and extraction of cholesterin and lecithin from the envelopes of the blood-corpuscles, yet a more extended research may show that it is only the modification of these substances by ether, and not their solution, that is to be looked upon as the cause of ether-laking.

THE INFLUENCE OF CHLOROFORM ON INTRAVITAL STAINING WITH METHYLENE-BLUE.

By C. A. HERTER AND A. N. RICHARDS.

THE object of this note is to record very briefly some results obtained by the use of intravital intravenous infusions of methylene-blue in rabbits which were rendered anæsthetic by the use of chloroform inhalations. These intravital infusions were made in twenty rabbits and the various findings were compared with the findings in control animals subjected to the same procedure, a control experiment being always carried on simultaneously with a chloroform experiment.¹ The methods of intravenous infusion closely resembled that used in the study of the effects of cold (*Journal of Physiology*, Sept. 1, 1904). The object of the experiments having been to determine the existence of differences in the behavior of the chloroformed and normal animals with respect to their action on methylene-blue, it was important to find the conditions of infusion which best exhibit these differences. It was found that the infusion of 25-30 c.c. of a 0.33 per cent solution of methylene-blue, at the rate of 1 c.c. per minute, into rabbits weighing about 1500 gms. usually gives the most pronounced differences. The animals were usually killed about ten minutes after the close of the infusion.

It should be understood that experiments conducted under these special conditions represent only one stage or phase of the action upon the infused methylene-blue. Very different results, some of them instructive, are obtained by using larger or smaller infusions, but under these circumstances one is apt to lose the differences almost regularly noticed under the conditions mentioned above.

In the majority of the experiments the chloroformed animals were rendered anæsthetic during a period of about one hour before the

¹ It is essential to carry on the two observations under conditions as nearly as possible alike. The rabbits should not only be about the same weight, but of the same color, since gray, white, and black rabbits present physiologic differences. In our experiments gray rabbits were generally used.

beginning of the infusion of methylene-blue, and were kept under the anæsthetic up to the time of death. It was found that the duration and depth of the anæsthesia considerably influenced the results, a slight anæsthesia leading to less pronounced deviations from the normal than a deep one. Many experiments proved failures, owing to death from chloroform.

Besides comparing the appearances of the organs in the normal and chloroformed animals, we have in numerous instances made observations on the amount of methylene-blue present in the different parts as methylene-blue, leuco-methylene-blue, or paired leuco-methylene-blue.¹ These observations proved helpful in the interpretation of the appearances at autopsy.

In addition to the experiments with chloroform, we made a smaller series with animals anæsthetized with ether. The results noted in these animals were similar to those noted in the case of the chloroformed animals, but were upon the whole less pronounced. It appears to us very desirable that a careful comparison of the effects of ether and chloroform should be carried out upon more highly organized animals than rabbits (dogs, monkeys).

The blood.—As in these experiments the methylene-blue is introduced intravenously, one of the first questions that arises relates to the blood's content of dye. It was found that in about one-half the experiments the serum of the chloroformed animals was bluer on separation from the clot than the serum of the controls. In most of these instances the addition of hydrogen peroxide confirmed the presence of a greater quantity of methylene-blue (or leuco-methylene-blue) in the serum of the chloroformed animals. Furthermore, in a majority of the experiments, on boiling the serum with dilute hydrochloric acid and subsequently oxidizing with peroxide, it became clear that the blood of the anæsthetized animals contained more paired leuco-methylene-blue than the blood of the controls. In about one-fifth of the experiments the control sera showed more methylene-blue (and paired leuco dye) than the chloroformed sera.

It is thus evident that in chloroformed animals the blood is apt to retain its methylene-blue longer than is the case with the blood of the control animals.

The muscles.—The muscles of the control animals were found in general to contain more methylene-blue than the muscles of the

¹ We have not yet been able to identify the substance which pairs in the liver with leuco-methylene-blue.

chloroformed animals. In ten of seventeen experiments in which the skeletal muscles were examined, there was more blue in the controls, in six of the experiments the bluing was the same in both animals, and in only one instance did the muscles of a chloroformed animal contain more dye. Observations on the color of the pectoral muscles during life gave more variable results. The heart of the chloroformed animals took the deeper stain in twelve experiments, in three cases the hearts were equally stained, and in three cases the chloroform hearts were most stained.

The kidneys.—The appearances in the kidneys correspond closely to the condition of the urine. In a large majority of all the experiments little or no methylene-blue was found in the usually scanty urine of the chloroformed rabbits. In these cases the medullary portion of the kidney contained very little of the dye. In the control animals the blue found its way readily into the urine, both as methylene-blue and as the paired leuco-substance. The kidneys in these cases were deeply stained in the medullary as well as in the cortical portions. It should be noted that in a majority of instances the cortical portions of the chloroform kidneys were more deeply stained than the same portions in the control animals. The chloroform kidneys almost regularly showed some congestion in the boundary zone, and in a number of instances treatment with hydrogen peroxide gave the impression that the process of reduction had been more active in those kidneys than in the controls.

The liver.—In sixteen out of twenty-one cases the livers of the chloroform animals were congested. When observed during life or immediately after death the livers of the chloroform animals were seen to be red, whereas the livers of the control animals were purple or blue-green. In many instances the liver extract from the chloroform animals contained more methylene-blue (either as blue or as the leuco compound) than the control livers;¹ the conditions were reversed in a much smaller group of cases. On the other hand, the control and chloroform livers were alike as regards the amount of paired leuco-methylene-blue present.

The bile presented a contrast to the liver in respect to the dye-stuff, for it was found that the excretion of blue, leuco-blue, and of paired leuco-blue (usually very scanty) occurred more freely in the case of the control livers than in the case of the companion livers

¹ This result may be due to the increased amount of methylene-blue in the blood.

from chloroformed animals. In other words, the chloroform exerted a retarding influence on the passage of the dye into the bile.

The pancreas. — In thirteen experiments the pancreas when first exposed was bluer in the control animal than in its anæsthetized fellow, in three cases the color was the same, and in two the chloroform pancreas was more deeply stained than the control. In the six cases in which hydrogen peroxide was applied the control pancreas darkened little or not at all, while the organs of the chloroformed rabbits were much deepened, usually becoming as blue as the pancreatic glands of the controls.

The suprarenals. — On exposure to the air or on oxidation with hydrogen peroxide more blue was found in the chloroform suprarenals than in the controls in fourteen cases out of twenty examined. The color was usually most abundant in the medullary portion. Our observations lead us to think that these differences in the receptivity of the suprarenals are readily masked by a slight excess of infused dye.

The brain. — The vessels of the pia mater were found to be slightly or moderately congested in nearly all the anæsthetized animals. In fourteen experiments the chloroform brains contained more blue than the control organs. In four instances the brains were about equally stained. In one case the control brain had the deepest color on oxidation.

The stomach and intestines. — Although the appearance of the mucous membrane was usually the same in chloroform and control rabbits (owing to post-mortal reduction) the application of hydrogen peroxide showed that there was usually more blue in the mucous and muscular coats of the chloroformed animals than in the corresponding structures of the control animals.

The spleen. — The spleens showed no important differences, but the organs of the control animals generally contained a little more of the dye than the organs of the anæsthetized animals.

The experiments which form the basis of this note were made especially with a view to learning whether under the influence of chloroform anæsthesia there is any deviation from the normal distribution or reduction of methylene-blue. In respect to the question of the reducing action of the chloroformed rabbits our experiments do not permit us to draw conclusions. In a few cases, in observations especially designed to permit a comparison of the reducing activities

of the gray substance of the brain in normal and anaesthetized animals, there was evidence of diminished reduction on the part of the chloroformed brains. Many more experiments will be necessary in order to settle this not unimportant point. The difficulties are greater here than in the case of animals subjected to cold, as the variations from normal are far slighter. In the instances where the pancreas was treated with oxidizing agents the behavior of the gland suggested an increased reducing activity (possibly coupled with disturbed oxidations) in consequence of chloroform intoxication, but the evidence at present possessed is inconclusive.

We come upon safer ground when we consider the question of the distribution of the infused methylene-blue. The dye is normally excreted by the kidneys and to a less extent by the liver and gastro-enteric mucous membrane. Chloroform impairs the excretory action both of the liver and the kidneys, and in consequence of this the blood holds the dye longer than it should and in greater concentration. It seems reasonable to believe that the increased concentration of the blue in the blood is the main factor in bringing about the moderate increase of blue observed in the brain and in the medulla of the suprarenal gland. This probably also offers the best explanation of the more abundant passage of the dye into the digestive tract. The muscles, however, appear to be independent of this greater concentration of dye in the blood, for it is the muscles of the controls which are usually most deeply stained. We have as yet found no satisfactory explanation of this behavior of the voluntary muscles and heart.

The appearances at autopsy were not so uniform in our experiments as one might perhaps infer from the undetailed statement which has been made. It was evident that normal individual peculiarities play a part in the distribution of blue in some instances. For example, in animals with small livers or livers incapable of taking up the usual proportion of the infused blue, the dye generally passes in increased amount into the liver. But it is possible that in some of these cases the relationship is the reverse of that just stated and that little blue sometimes goes to the liver because of an uncommon avidity of the muscles.

In a series of experiments upon rabbits in a state of ether narcosis (comparable in duration with the periods of chloroform narcosis) it was found that the dye was distributed as in the case of the chloroform experiments with two exceptions: first, that the hearts of the ether-

ized animals were less stained than those of the chloroformed animals, and, secondly, that less blue passed into the gastro-enteric tract.

It is our intention to report more fully upon the influence of ether and chloroform upon the fate of dyes, and to add observations on the influence of acetone, alcohol, ethylene chloride, and other narcotizing and anæsthetic agents. The interest of the present study seems to us to centre in the demonstration that chloroform intoxication is attended by widespread disturbance of function.

HYDROLYSIS OF SPLEEN-NUCLEIC ACID BY DILUTE MINERAL ACID.

By P. A. LEVENE.

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THE end cleavage products of nucleic acid are phosphoric acid, purin-bases, pyrimidin bases, and carbohydrates. Some of these components enter the molecule of the acid in a very loose combination. On the contrary, other constituents are so intimately combined that it is impossible to obtain them without breaking up the entire molecule of the acid. This involves the use of powerful reagents, as strong acids or strong alkalies. Under such conditions the original constituents of the nucleic acid undergo various changes, so that their exact composition remains unknown. Thus of the carbohydrates it is known that one yields levulinic acid, and the other furfural. The mother substance of the pyrimidin bases is also not known. Much less satisfactory is the knowledge of the grouping of the various components within the molecule. It is certain that some constituents can be easily removed by means of dilute acid, and that the remaining part is more resistant. This remainder is looked upon as the nucleus on which the other components are built to form the complex nucleic acid. Purin bases are those that are removed by comparatively mild treatment. The hypothetical remainder was named by Schmiedeberg¹ nucleotin phosphoric acid. It is supposed to contain less nitrogen and more phosphorus than the original acid. Previous to Schmiedeberg, a substance with similar properties was described by Kossel and Neumann,² and was designated as thyminic acid. Osborne and Harris³ also attempted

¹ SCHMIEDEBERG: *Archiv für experimentelle Pathologie und Pharmacologie*, 1900, xliii, p. 57. See also ALSBERG: *Ibid.*, 1904, li, p. 238.

² KOSSEL and NEUMANN: *Zeitschrift für physiologische Chemie*, 1896-97, xxii, p. 74. See also NEUMANN: *Archiv für Physiologie*, 1898, p. 374.

³ OSBORNE and HARRIS: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 85.

to obtain the primary cleavage products of nucleic acid. They found that on boiling their triticonucleic acid with a 2 per cent solution of sulphuric acid, a substance poorer in phosphorus and in nitrogen, and also in the mother substance of furfural, could be obtained. The substances obtained by different workers differ greatly in their composition. This difference was caused partly by the fact that alcohol was employed for precipitating the substance from the end-products of the hydrolysis of these acids.

Realizing the difficulty in obtaining the intermediate products of hydrolysis in a sufficient degree of purity, it was decided to subject nucleic acid to hydrolysis with dilute acids, and to subject the substances thus obtained to further decomposition. The work had also another aim; namely, the comparison of acids derived from different tissues. Spleen-nucleic acid was employed for the present experiment. It was prepared by the writer's second process. This method differs from the first in that no alcohol is used for precipitating the substance.¹

The substance obtained in this manner has a fairly uniform composition, as is seen from the following analysis of three different samples obtained at different times.

Sample I.—Supposed to have been the free acid, but contained considerable mineral substance.

0.2970 gm. of the substance was employed for a Kjeldahl nitrogen estimation. It required 25.50 c.c. $\frac{N}{10}$ H_2SO_4 ; N = 12.05 per cent.

0.355 gm. of the substance gave 0.098 $Mg_2P_2O_7$; P = 7.72 per cent. The substance contained 29 per cent of ash.

Sample II.—*Sodium salt.* 0.2550 gm. of the substance was employed for a Kjeldahl nitrogen estimation. It required 19.20 c.c. $\frac{N}{10}$ H_2SO_4 ; N = 10.54. 0.2520 gm. of the substance gave 0.0620 gm. of $Mg_2P_2O_7$; P = 6.79.

The substance contained 34 per cent of ash.

Sample III.—*Copper salt.* 0.2250 gm. of the substance was employed for a Kjeldahl nitrogen estimation. It required 17.10 c.c. of $\frac{N}{10}$ H_2SO_4 ; N = 10.54.

0.341 gm. of the substance gave 0.0940 gm. of $Mg_2P_2O_7$; P = 8.70 per cent.

¹ In Hammarsten's Text Book, as well as in Burian's article in the *Ergebnisse der Physiologie*, only the first process is referred to; no mention is made of the method described in the writer's second communication, "On the Preparation and on the Analysis of Nucleic Acid." However, it is not certain whether the sub-

The substance contained 33.7 per cent of ash.

Calculated for the free acid, the substances had the following composition:

Sample I.	II.	III.
N = 13.48	13.17	12.65
P = 8.67	8.49	8.70

A freshly prepared sample of nucleic acid not yet dry was taken up with a 2 per cent solution of sulphuric acid and heated in autoclave for 4 hours at $t^{\circ} = 100-125^{\circ} \text{C}$. It was then filtered, the filtrate brought to a definite volume, and a nitrogen estimation made. It was found to contain 10.9 gms. of nitrogen. The residue was washed, filtered, extracted with alcohol and ether, and dried. It then weighed 27 gms. containing 3.25 gms. of nitrogen. Thus about 109 gms. of nucleic acid were employed for the experiment.

The filtrate was employed for the analysis of purin bases. It was concentrated to a small volume and rendered alkaline with ammonia. A precipitate resulted (precipitate I), which was removed by filtration on a suction funnel (filtrate I). Precipitate I was redissolved in dilute sulphuric acid and reprecipitated with ammonia. The substance proved on analysis to contain only 16 per cent of nitrogen. It was therefore redissolved in dilute sulphuric acid and the guanin removed by means of an ammoniacal solution of silver chloride. The silver was then removed by hydrochloric acid, the filtrate from silver chloride was concentrated under diminished pressure, rendered alkaline by means of ammonia, heated, and the free guanin filtered while the mother liquid was still hot. The free base was transformed into the sulphate. 2 gms. of the sulphate were obtained.

0.1250 gm. of the substance (air dry) was employed for a Kjeldahl nitrogen estimation. 28.80 c.c. of $\frac{N}{16} \text{H}_2\text{SO}_4$ were required.

For $(\text{C}_5\text{H}_5\text{N}_5\text{O})_2, \text{H}_2\text{SO}_4 + 2\text{H}_{20}$:

Calculated:
N = 33.21 per cent.

Found:
32.24 per cent.

The mother liquid of guanin sulphate was added to filtrate I, and the purin bases were precipitated by an ammoniacal solution of silver

stances obtained by the two methods are identical. All the work of the writer on the hydrolytic cleavage of nucleic acids was done on substances obtained by the second process. See LEVENE: *Zeitschrift für physiologische Chemie*, 1901, xxxii, p. 541, and 1902-03, xxxvii, p. 402.

chloride. The silver was removed in the usual manner and adenin precipitated by means of sodium picrate. 30 gms. of the substance were obtained. Part of it was dissolved in boiling water containing just sufficient sodium hydrate to effect solution. Hydrochloric acid was added to neutralize the soda, and the solution was decolorized by means of charcoal. It was then filtered and placed in a thermostat to cause slow crystallization. The melting point of the substance was 282° C.

The mother liquid of adenin picrate was acidulated with hydrochloric acid and extracted with ether. It was then rendered ammoniacal and treated with an ammoniacal solution of silver chloride. The precipitate was decomposed in the usual manner, and the solution of purin bases thus obtained was treated according to the method of Kruger and Solomon. The part insoluble in water did not contain any xanthin.

The soluble part formed a picrate, which, however, was not hypoxanthin, since it did not form an insoluble nitrate. The picrate weighed 1.5 gms. and was either adenin or guanin.

The residue was extracted with alcohol and ether, and dried. It weighed 27 gms. and had the following composition:

0.1828 gm. of substance were employed for a Kjeldahl nitrogen estimation. It required 11.6 c.c. $\frac{n}{10}$ H_2SO_4 ; N = 8.83 per cent.

0.480 gm. of the substance gave 0.148 gm. of $\text{Mg}_2\text{P}_2\text{O}_7$; P = 8.62 per cent.

The substance contained 39.0 per cent ash and 4 per cent water.

The residue had a fairly constant composition, as is seen from the fact that repeated precipitation does not affect its composition.

In another experiment a quantity of nucleic acid approximately equal to that used for the first decomposition was heated in autoclave in a 2 per cent solution of sulphuric acid at a temperature of $100-125^{\circ}$ C. for 4 hours.

The residue obtained on this treatment was dissolved in a potassium hydrate solution, filtered and reprecipitated by means of hydrochloric acid. The operation was repeated three times, and the substance finally was extracted with alcohol and ether, dried and analyzed.

0.2100 gm. of the substance was employed for a Kjeldahl nitrogen estimation. It required 13.05 c.c. $\frac{n}{10}$ H_2SO_4 ; N = 8.7 per cent.

0.420 gm. of the substance gave 0.1189 gm. $\text{Mg}_2\text{P}_2\text{O}_7$; P = 7.95 per cent.

The substance contained 48.10 ash.

Calculated for free acid, the two residues had the following composition:

Subst. I.	Subst. II.
N = 12.25 per cent.	12.42 per cent.
P = 11.32 "	11.35 "

This seems to indicate that the substance is not a mixture of various primary cleavage products of the nucleic acid. Though the substance is still insoluble in dilute mineral acids, it may be regarded as a depolymerization product of the original acid, since it dissolves more readily in alkalies, and the alkaline solution filters well, not showing any tendency toward gelatinization.

With the primary cleavage products obtained by other workers it compares as follows:

Thyminic acid (Kossel and Neumann).	Heminucleic acid (Schmiedeberg, also Alsberg).	Osborne and Harris.	Levene.
N = 9 per cent.	10.73 per cent.	11.05 per cent.	12.33 per cent.
P = 12.08 "	10.74 "	10.00 "	11.33 "

From the standpoint of its physical properties the substance resembles the nucleothyminic acid of Neumann.

In order to ascertain the relation of the substance to the original acid, its decomposition products were obtained. As already stated, nucleic acid consists of purin bases, pyrimidin bases, a substance giving levulinic acid and one giving furfurol.

Purin and pyrimidin bases.—25 gms. of the dry residue were decomposed in an autoclave with a 10 per cent solution of sulphuric acid for two hours at a temperature of 100–125° C. The sulphuric acid was removed by baryta water, the filtrate was rendered ammoniacal and treated with an ammoniacal solution of silver chloride. A very small flocculent precipitate of purin bases was obtained. No further separation of the bases was undertaken in view of the small yield of the substance. To the filtrate sufficient hydrochloric acid was added to remove the silver. The filtrate from silver chloride was made alkaline by means of baryta water, and the excess of barium was removed in the usual manner. The pyrimidin bases were precipitated by the Kossel-Jones method. The precipitate consisted of thymine and cytosine.

There was about 0.200 gm. of pure thymine and 0.225 gm. of cytosine picrate.

However, the yield of pyrimidin bases was much smaller than would be expected from 100 gm. of spleen-nucleic acid. Therefore part of the pyrimidin bases or their mother substance must have been in the original sulphuric acid solution.

Thus the substance contained very little purin bases as compared with the original acid, and did not contain all the pyrimidin bases that could be obtained from 100 gms. nucleic acid, and in fact much less than could be obtained from an equal weight of nucleic acid. The nature of the carbohydrate was examined next.

A furfural estimation was made on the sodium salt of the nucleic acid and on the residue.

2.3680 gms. of the sodium salt gave 0.0237 gms. of the phlorglucid.
1.6900 gms. of the residue gave 0.005 gm. of the phlorglucid.

Thus it is seen that the substance yielding furfural is nearly totally broken up by the treatment with a 2 per cent solution of sulphuric acid, and that it had to be classified among the substances in loose combination with the nucleus of the acid.

In order to ascertain whether the residue contains a carbohydrate of the hexose group, 17 gms. of the dry residue were taken up in 100 c.c. of a 25 per cent solution of sulphuric acid, and heated on a boiling water bath. The cold solution was extracted with ether and the ether removed by distillation. The residue was taken up in ether. It gave the color test for levulinic acid, and the silver salt of levulinic acid was obtained in the usual manner. Once recrystallized, it had the following composition:

0.1170 gm. of the substance gave 0.0570 gm. Ag.

For $C_6H_7O_3$ Ag.:

Calculated:
Ag = 48.43 per cent.

Found:
48.71 per cent.

The yield of levulinic acid was larger than could be expected from a corresponding quantity of nucleic acid. Thus it seems probable that the hexose is a part of the "ground substance" (*Grundsubstanz*) of the nucleic acid, and that it is present in the molecule in form of a very stable polysaccharid.

Tryptic digestion of nucleic acid.—No satisfactory results thus far were obtained by this method. About 30 gms. of the nucleic acid were dissolved in a 5 per cent solution of sodium carbonate, and the solution was diluted with nine volumes of water, 2 gms. of a very active trypsin (*trypsinum purissimum* Grubler,—it was used for

other work in the laboratory and found very active) were added. The solution was placed in thermostat. Toluol and chloroform were added to prevent bacterial growth.

After one week a part of the solution was taken and the nucleic acid precipitated from it by means of hydrochloric acid. It was transformed into its sodium salt. After twenty days another part of the solution was taken for experiment and treated in the same manner.

The original substance and the two digestion products had the following composition:

	N.	P.
Original substance.	13.17 per cent.	8.49 per cent.
First digestion.	12.55 "	9.59 "
Second digestion.	15.06 "	8.40 "

Thus there is noticed first an increase of phosphorus by a breaking off of the purin bases, followed later by cleavage of the phosphoric acid.

The relative proportions of the furfural obtained from the original substance, and from the first product of digestion, were as follows:

Original substance: 2.368 gms. of the substance gave 0.0237 gm. of furfural phlorglucid.

First digestion product: 2.400 gms. of the substance gave 0.0180 gm. of furfural phlorglucid.

The cleavage by means of trypsin, as seen from this experiment, was very slow. It is probable, therefore, that within the tissues the cleavage of nucleic acid is caused by a special enzyme.

I wish to express my indebtedness to Prof. R. H. Chittenden, under whose control this investigation was carried out. Doctor L. B. Stookey kindly assisted me in the analytical part of the work, and I wish to express my thanks to him.

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A STUDY OF THE EFFECTS OF CERTAIN STIMULI, SINGLE AND COMBINED, UPON PARAMÆCIUM.

By ELIZABETH W. TOWLE.

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INTRODUCTION.

*Cause
liquefaction*
*Cause
coagulation*
THE factors which produce structural changes in protoplasm have been divided, according to their effects, into two main classes: (1) those which bring about liquefaction, (2) those which cause coagulation. Among the former are included (a) temperatures above normal but below the "critical point;" (b) non-electrolytes such as cane-sugar and urea, in solutions whose osmotic pressure is much below that of the protoplasm, *i. e.*, solutions hypotonic to protoplasm; (c) all electrolytes in which the action of the anion is predominant¹ over that of the kation. Factors leading to coagulation are: (a) temperatures below normal and those above the critical point; (b) non-electrolytes in solutions whose osmotic pressure exceeds that of the protoplasm; (c) electrolytes in which the action of the kation predominates; and also (d) all electrolytes in such high concentrations that osmotic effects prevail over ionic.² The purpose of the work described in the present paper was to test the validity of this classification by a study of effects produced when factors, apparently antagonistic, act simultaneously. If an agent, physical or chemical, leads to the absorption of water and to liquefaction, and another to loss of water and coagulation, it might be expected that simultaneous application of these agents in certain proportions would produce, if not a complete neutralization of each effect, at least a reduction in its rate or intensity. The problem undertaken was a perfectly definite one, and a definite answer was expected. But, as the results will show, this was not obtained. It should be

¹ I use this term in the sense given by MATHEWS: This journal, 1904, x, p. 290.

² This is essentially the classification given by GREELEY: Biological Bulletin, 1904, vii, p. 3.

said, however, that there has been no effort to make the experiments exhaustive, and the author is not at present prepared to offer a theoretical explanation for the discrepancies observed. In fact, the results are valuable rather from a negative than a positive standpoint, for they emphasize strongly the necessity for extreme caution in drawing general conclusions with regard to the processes that take place in living cells.

METHODS AND DIFFICULTIES INVOLVED.

As an object of investigation, Paramœcium has always proved most attractive to students of protoplasmic reactions. It was again chosen here, partly because it is so easily obtained in large numbers, but mainly because it has been shown to be very responsive to slight changes in environment — chemical, thermal, electric, etc.

Cultures. — The species studied varied somewhat, as a number of different cultures were used, and the forms were not always the same in adjoining aquaria. The method of keeping the cultures was similar to that employed by Greeley,¹ small pieces of bread and fresh tap water being added from time to time. Many were kept in this way without trouble during the winter. Such old cultures were always acid to phenol phthalëin. Occasionally a dying culture was allowed to dry out completely, and was then made active again by the addition of water and bread. This happened most frequently during the spring months, April to June. New cultures always remained alkaline or neutral for some time after being started, but later they also became acid. Thus opportunity was given to study Paramœcia from the three kinds of culture media.

Effect of distilled water. — The earliest experiments undertaken proved very uncertain, as Paramœcia died uniformly with vacuolation in all strengths of the reagents employed and also in distilled water, this fact making it impossible to differentiate the effects of the dissolved substance from those of the solvent. The water was furnished from the chemistry department and was thought to contain some impurity. Experiment showed, however, that two further distillations made in glass rendered it perfectly harmless, and Paramœcia often lived in it without food for weeks at a time. All solutions used subsequently were therefore made up with this doubly distilled water. In order to avoid the possibility of variations due to

¹ GREELEY: *Loc. cit.*

slight differences in the salts used, these were obtained chemically pure, and a quantity of each was recrystallized from purified water.

The statement that certain organisms will live indefinitely in pure water has previously been made by Jennings,¹ Locke,² Loeb,³ and others. Greeley⁴ found, on the contrary, that *Paramœcia* liquefy in water alone. As the water that he used was not redistilled in glass, it seems probable that it contained some impurity which affected the protoplasm. If this was the case, the results which he obtained need reconsideration, and one factor inducing liquefaction disappears from the list; *vis.*, non-electrolytes in hypotonic solution. This I believe to be the case, as in no instance have I seen liquefaction caused by such solutions when the solvent was pure water.

Washing. — The problem next arose as to the amount of washing necessary before *Paramœcia* were brought into the solutions to be studied. Jennings has recorded the observation that for a few days after immersion in distilled water *Paramœcia* show extreme sensitiveness to all reagents employed, but this diminishes after a sufficient time has elapsed. Long washing was, however, not desired for the purposes of these experiments, for after it any comparison between the behavior of animals from acid and from alkaline cultures becomes impossible. There is an objection also to introducing *Paramœcia* directly into solutions without washing, for there are necessarily variations in the chemical nature of the cultures used. Not only are there differences in the amount of acid or alkali present, but other substances in solution vary in different cultures at the same time, and also in the same culture at different times. As it was impossible to eliminate both of the above difficulties at once, it became necessary to determine in how far the presence of small traces of the original culture modify the behavior of the organism toward different reagents.

The first experiments performed were of particular interest, as the results were unique. *Paramœcia* from a neutral culture were exposed to the action of hydrochloric acid. The solution was made by adding acid to water in the proportion of four drops of $\frac{m}{800}$ HCl to 5 c.c. H₂O. Some of the culture was diluted with two or three times its volume of water, and from this three drops containing *Paramœcia* were put

¹ JENNINGS: *Journal of physiology*, 1897, xxi, p. 258.

² LOCKE: *Ibid.*, 1895, xviii, p. 319.

³ LOEB: *This journal*, 1900, iii, p. 332.

⁴ GREELEY: *Loc. cit.*

at different intervals into 5 c.c. of acid. As a control, one drop was put into acid directly from the original culture. The results obtained were very striking. During the first five minutes after the addition of water the sensitiveness of the organisms increased rapidly, but it remained fairly constant throughout the rest of the experiment. Most conspicuous, however, was the fact that Paramœcia taken directly from the original culture lived for hours in a strength of acid in which, after short exposure to distilled water, they died very quickly.

Three days later the experiment was slightly modified, Paramœcia from the same culture being used. Instead of being first left in distilled water, a number were put directly into hydrochloric acid solution. At varying intervals some of these were then transferred to fresh hydrochloric acid solutions, the culture thus being diluted by acid and not by water. The results were similar; Paramœcia which passed directly from culture to acid lived for hours, while those which were transferred to fresh acid solution died uniformly in from two to five minutes. Evidently the presence of even exceedingly small amounts of the original culture sufficed in this case to inhibit the injurious action of hydrochloric acid. This was the more striking, as the culture, on titration with phenol phthalëin as indicator, was found to be neutral.

These results led me to repeat the experiments with Paramœcia from a number of different cultures, varying the amount of water added, the time of exposure, and the strength of hydrochloric acid employed. In no other case did I get results like the above, whether the culture tested was acid or alkaline. In the majority of instances there was a slight increase in sensitiveness after exposure to distilled water, but hydrochloric acid was fatal also to individuals from the original culture. The obvious conclusion is that in the culture first employed there was a substance which, when present in even small amounts, in some way was able to inhibit the action of hydrochloric acid in the strengths used.¹ It seems to me very important that this point should be borne in mind and careful control experiments made whenever one is investigating the reactions of Protozoa to dissolved substances. Only in case of uniformity in the behavior of individuals from many different cultures can we be sure that we are dealing with general laws, and not with specific phenomena due to certain

¹ That certain substances may produce this effect will be seen in later experiments, pp. 231-233.

conditions in the media from which the organisms are taken. The suggestion has been made and should, I think, be followed out, that in the study of Protozoan reactions a standard culture medium and a standard food organism be used, as is done in the study of bacteria. Only in this way will it ever be possible to correlate with certainty the observations of different investigators. No such medium is as yet available, and the experiments to be described are, therefore, open to objection. They have been repeated, however, on *Paramœcia* from a number of cultures, and the results have proved uniform in the majority of cases.

Experiments were next made to determine the effect of washing upon the reaction of *Paramœcia* to substances other than hydrochloric acid. These substances are enumerated on page 225. Here, as before, a slight increase in sensitiveness followed exposure to distilled water, but there was no radical difference even when considerable amounts of the culture were present. Similarly, the reactions of *Paramœcia* from acid and alkaline cultures were compared. The character of the response was the same in either case, although there was a slight difference in sensitiveness, *Paramœcia* from acid cultures usually dying more quickly in hydrochloric acid, sodium hydrate, and sodium citrate, those from alkaline cultures in sodium chloride, sodium sulphate, and calcium chloride. No great significance was, however, attached to this, for, as will be shown later, such variations are to be expected. The following method of washing was fixed upon as least objectionable, and was used uniformly in all further experiments. To a given volume of culture an equal volume of water was added, and the *Paramœcia* allowed to remain for five minutes; then two to four drops of this preparation were transferred to 5 c.c. of the solutions to be used.

Tolerance.—Finally, the question arose as to whether it was better to study the effects of weak solutions over long periods or of stronger solutions over short periods. This was soon answered, for it was found that when *Paramœcia* survived in a given solution beyond a certain limited time (varying with the substance used), a condition of tolerance was reached, and they continued to live, often for days. The main stress was therefore laid upon reactions which occurred within periods of one to two hours, or even less.

EFFECT OF LOW TEMPERATURE AND DIFFERENT SOLUTIONS
UPON PARAMŒCIA.

The following are the stimuli whose effects were studied :

1. Temperatures below normal : $0^{\circ} - 8^{\circ}$ C.
2. Electrolytes with predominant anions :

NaOH	$\frac{m}{100} - \frac{m}{800}$
Na acetate	$\frac{m}{10} - \frac{m}{50}$
Na ₃ citrate	$\frac{m}{100} - \frac{m}{1000}$
NaCl	$\frac{m}{10} - \frac{m}{50}$
Na ₂ SO ₄	$\frac{m}{20} - \frac{m}{70}$

3. Electrolytes with predominant cations :

HCl	$\frac{m}{1000} - \frac{m}{10000}$
CaCl ₂	$\frac{m}{10} - \frac{m}{50}$

4. Non-electrolytes in strong solutions :

Cane-sugar	$\frac{m}{2} - \frac{m}{10}$
Urea	$\frac{m}{2} - \frac{m}{10}$

Of these, the second group alone has been considered as leading to the absorption of water and liquefaction of protoplasm, while groups 1, 3, and 4 bring about loss of water and condensation or coagulation. If this view is correct it should be possible to nullify, or at least to reduce the effect of group 2 by combining with it stimuli from 1, 3, or 4. On the other hand, a combination of stimuli from 1, 3, or 4 should be additive in its effect, the protoplasm responding by coagulation more rapid than that produced by any single agent.

In order to interpret correctly the results obtained by combining stimuli, a careful study was first necessary of the effects produced by each stimulus acting alone. This, while yielding results which agreed in the main with the theory, brought out also a number of unexpected variations, and facts not easy to correlate, upon which I wish to lay some stress.

Effects of single stimuli. — 1. *Low temperature.* The results of exposure to low temperatures were uniform and agreed with those described by previous workers. The method was slightly different from that usually employed ; for, instead of gradually cooling the medium in which Paramœcia were living, I dropped them suddenly into water of the desired temperature. This was done in order to eliminate

the possibility of gradual acclimatization. The results are the same with either method. Paramœcia when cooled become torpid, the degree of torpidity increasing the lower the temperature used. Near 0° C. all movement ceases rapidly, and the animals become quite rigid. With increase in torpidity comes an increase in opacity of the protoplasm and possibly slight shrinkage.

2. *Solutions.* The first conspicuous variation was seen in studying the behavior of Paramœcia in different solutions. The sensitiveness of the organisms was found to change greatly from one time to another, so that, while in a given strength of a certain substance they lived indefinitely at one time, they often died in it very quickly a few days later. This fact is so striking that it makes of little or no value accurate statements as to the absolute strength of solutions used. For example, I have seen Paramœcia at one time live for hours at room temperature in $\frac{m}{800}$ NaOH solution, while again they would liquefy within half an hour in the same solution. Nor are the changes in sensitiveness necessarily correlated with changes in the reaction of the original culture. For the differences were often very considerable within periods as short as eighteen to twenty-four hours, when titration with phenol phthaleïn showed practically no change in the acidity or alkalinity of the medium. For this reason, in cases where it was necessary to know the strength which would just kill in a given time, control experiments had to be made daily. Here again we are obviously dealing with unmeasured slight changes either in the Paramœcia themselves or in the chemical nature of the medium in which they live.

Of the action of electrolytes, it may be said in general that those with predominant anions led to liquefaction of the protoplasm, those with predominant kations to its condensation. But here again variations appear immediately. Those occurring in the former group of substances are most conspicuous and are of two kinds; first, differences—at a given time—in the process of liquefaction as produced by different substances; second, differences in the mode of action of the same solution at different times.

As an instance of the first type may be cited the effect upon Paramœcia from a single culture, of equally fatal doses of sodium hydrate and sodium citrate. Such doses may be considered as in a sense physiologically equivalent.¹ If at a certain time it is ascertained, for

¹ Such equivalence, for reasons explained above, must be determined afresh for each experiment, as the sensitiveness of Paramœcia for different substances varies without apparent regularity.

example, that solutions of $\frac{m}{200}$ NaOH and $\frac{m}{400}$ Na_3 citrate kill Paramœcia with equal rapidity, the steps in the process of disintegration are seen to be quite different in the two solutions. Paramœcia in sodium hydrate undergo extensive vacuolation, swell rapidly, especially at the posterior end, and assume a peculiar pear-shaped form which has been often described and which I have never seen in any other reagent. Later the wall bursts and all trace of the organisms disappears. Those in sodium citrate, on the other hand, swell much less, but huge watery vesicles may appear which project from the sides and later burst, their contents mingling with the water. The Paramœcia thus lose their form and eventually break up, leaving patches of protoplasm scattered throughout the solution, but not disappearing altogether until perhaps many hours later.¹

The second type of variation may be illustrated by the action of physiologically equivalent solutions of, *e.g.*, sodium citrate, on different days. At one time the dominant effect may be a rounding up and vacuolation of the protoplasm, whereas again only small vacuoles form, their place being taken by large protruding vesicles. Similar differences may be seen in solutions of sodium acetate and of sodium chloride. The character of the vesicles also varies at different times and in different solutions. Sometimes they are large, slightly refractive, and filled with a substance which mingles readily with water. Again they are much smaller, more refractive, and contain a substance which is slightly if at all soluble in water.

A study of solutions containing predominant kations brings us to another type of variations which it is of importance to note; namely, the appearance of secondary changes in the protoplasm, differing in character from those usually described.

The effect of fatal doses of hydrochloric acid is well known. When exposed to them, Paramœcia become torpid and opaque; there is a slight decrease in size, and finally the organisms become nearly spherical and quite rigid. An opposite process, however, often follows shrinkage. The protoplasm, while remaining denser than that of normal Paramœcia, becomes swollen and clearer than before, and in a few cases I have even seen vesicles form on the surface. A certain amount of disintegration follows this phenomenon. My first thought was that this change never occurs until after the death of

¹ This process resembles closely that described by LOEB and HARDESTY for Paramœcia exposed to the action of carbon dioxide or nitrogen: *Archiv für die gesammte Physiologie*, 1895, lxi, p. 583.

the animals;¹ but this is not always true, for I have seen *Paramœcia* much swollen long before ciliary movement has ceased, while the contractile vacuoles are still pulsating normally.

In calcium chloride something more than simple coagulation of the protoplasm occurs, for disintegration like that described for hydrochloric acid is often seen here. During one series of experiments I used very large *Paramœcia* from a culture that was slightly alkaline. Upon exposure to calcium chloride a number of individuals became clouded and swollen at the anterior end. The wall finally burst and the animals went to pieces; but the protoplasm did not diffuse through the water as does that of *Paramœcia* exposed to sodium hydrate.

These secondary changes become most conspicuous when the fourth group of stimuli is studied; *viz.*, solutions of cane-sugar and urea. It was found that in the great majority of cases *Paramœcia* lived indefinitely without change of form in solutions weaker than $\frac{m}{8}$, and I have even known them to live for days in solutions as strong as $\frac{m}{6}$. Observation of the phenomena taking place in fatal solutions showed first a marked shrinkage of the organism, due no doubt to loss of water, while movement gradually ceased as on exposure to low temperature. After a varying length of time, and occasionally before movement had entirely ceased, another process began. The organism became swollen and rounded, the cell wall gave way in one or more places and often seemed to melt altogether, while the protoplasm beneath it mingled, to a certain extent, with the water.

In the case of urea the secondary action was very powerful, so much so that only in strong solutions ($\frac{m}{2}$) did the primary effect of shrinkage appear at all, and then for only a few moments. Very soon after immersion in $\frac{m}{4}$ urea, a slight clouding appeared at the anterior end, and the animals began to swell. This continued for some time after movement had ceased; the *Paramœcia* finally burst, and the contents were to a considerable extent diffused through the water.²

Two possible explanations for these secondary effects have sug-

¹ Changes of this sort *after death* have been previously noted by DALE: *Journal of physiology*, 1901, xxvi, p. 291.

² As this paper goes to press my attention is called to the fact that urea acts as a weak base, and also enters most cells with great ease. This may prove to be the explanation of results described here and on pp. 230-231.

gested themselves: (1) changes (chemical?) may take place in the protoplasm itself, leading to changes in its relation toward the surrounding medium and resulting in absorption of water; (2) alterations may occur in the permeability of the cell-membrane. It is probable that both these factors are involved.

A consideration of the results described above will show that in any attempt to neutralize one stimulus by another many factors will be involved which do not at first appear. The most important of these are: (a) the influence of acclimatization; (b) variations in sensitiveness and mode of reaction of Paramœcia due probably to (c) slight but important variations in the chemical nature of the cultures used; and finally (d) secondary processes occurring in the protoplasm which may be antagonistic to the primary and more obvious ones. In the experiments upon combined stimuli which follow, I shall, therefore, give an account only of general results, as, for reasons already explained, I lay but little stress upon the absolute data of any one experiment.

Effects of combined stimuli. — In studying the effect of low temperatures, the solutions to be tested were first cooled to the desired point and Paramœcia then dropped in, just as when distilled water alone was used. Controls were always kept at room temperature (19° – 22° C.). When any two opposing solutions were used, $2\frac{1}{2}$ c.c. of one were mixed with $2\frac{1}{2}$ c.c. of the other, each being, however, twice the strength of the control. This was done so that the relative amount of each substance per 5 c.c. of water should remain unchanged; *e. g.*,

$2\frac{1}{2}$ c.c. $\frac{m}{150}$ Na_3 citrate + $2\frac{1}{2}$ c.c. $\frac{m}{4}$ cane-sugar.

Controls: 5 c.c. $\frac{m}{300}$ Na_3 citrate; 5 c.c. $\frac{m}{8}$ cane-sugar.

The same effect was in some cases produced by adding a few drops of a strong solution of one substance to 5 c.c. of the other; *e. g.*,

5 c.c. $\frac{m}{8}$ cane-sugar + 3 drops $\frac{m}{10}$ KOH.

Controls: 5 c.c. $\frac{m}{8}$ cane-sugar + 3 drops H_2O .

5 c.c. H_2O + 3 drops $\frac{m}{10}$ KOH.

The following stimuli were used: (1) Low temperature *versus* (a) anions, (b) kations, (c) non-electrolytes; (2) non-electrolytes *versus* (a) anions, (b), kations; (3) kations *versus* anions.

1. *Low temperature.* The effect of low temperatures (2° – 10° C.) was tried upon all the solutions used with uniform results. In all cases where the cold was sufficient to influence the action at all, it retarded it. This is to be expected when the solutions used are liquefying agents, such as sodium hydrate or sodium citrate. But when Paramœcia are exposed to hydrochloric acid at a temperature of $+3^{\circ}$ C., if the main influence of each agent is to withdraw water from the protoplasm, an additive effect might be looked for. The fact that this is not obtained points to the conclusion that we are dealing not simply with loss of water, but also with certain chemical effects, which are due to the action of the acid, and are retarded by the lowered temperature.¹

2a. *Non-electrolytes versus anions.*

Cane-sugar + KOH.

Cane-sugar + Na_3 citrate.

Urea + KOH.

Urea + Na_3 citrate.

Cane-sugar was used in strengths from $\frac{m}{8}$ to $\frac{m}{10}$. In such solutions Paramœcia will generally live indefinitely. When cane-sugar was mixed with potassium hydrate the following results were obtained. The fatal effect of strong solutions of sugar was not affected by non-fatal doses of potassium hydrate. The absorption of water and liquefaction produced by fatal doses of potassium hydrate was greatly retarded, but not inhibited, by nonfatal doses of cane-sugar. If a just fatal dose of one was combined with a just fatal dose of the other, the resulting solution was equally fatal. The secondary effect of cane-sugar appeared in the fact that Paramœcia in solutions of cane-sugar containing amounts of potassium hydrate just below fatal went to pieces after death much more rapidly and completely than those in either substance alone.

When cane-sugar was opposed to sodium citrate analogous results were obtained. The swelling action of the citrate was retarded by sugar in nonfatal doses, while the fatal effect of strong solutions of sugar was not altered by a strength of citrate nearly fatal. In the later case, however, disintegration was more rapid than in citrate alone.

In urea,² we have a substance whose secondary action in strong

¹ Cf. p. 228.

² See note 2, p. 228.

solution so predominates over osmotic effects that the resultant is swelling rather than shrinkage of the protoplasm. Owing to this fact, it allies itself in its effects rather with the group of liquefying agents than with those causing coagulation. For this reason it is to be expected that its influence upon potassium hydrate and sodium citrate will be additive rather than inhibitory, and this is true in some cases. The effect was clearly additive when fatal doses of the two were combined. For example, in 5 c.c. $\frac{m}{2}$ urea + 3 drops $\frac{m}{10}$ KOH, the majority of Paramœcia disappeared within five minutes. In $\frac{m}{2}$ urea they were dead and beginning to go to pieces, while in 5 c.c. H_2O + 3 drops KOH the majority were alive. These latter disappeared after fifteen minutes. Subfatal doses of potassium hydrate did not affect the action of fatal amounts of urea, nor did subfatal urea solutions appreciably affect the liquefying action of fatal amounts of potassium hydrate. Similarly, a mixture of urea and sodium citrate was found to be more fatal than was either solution alone.

2b. *Non-electrolytes versus kations.* Observation of the peculiar liquefying action of urea led me to try the effect of opposing it with hydrochloric acid. This yielded very clear results. Subfatal solutions of urea were found to retard greatly the coagulating action of strong hydrochloric acid; *e.g.*,

- 5 c.c. $\frac{m}{4}$ urea + 2 drops $\frac{m}{100}$ HCl. Alive after 24 hours.
5 c.c. $\frac{m}{4}$ urea. Alive after 24 hours.
5 c.c. HO + 2 drops $\frac{m}{100}$ HCl. Dead in 5 minutes.

Fatal doses of acid did not prevent death in fatal solutions of urea, although liquefaction was delayed; in subfatal doses the acid had no effect.

3. *Kations versus anions.*

HCl + Na acetate.
HCl + Na_3 citrate.
 $CaCl_2$ + Na acetate.

Here unmistakable instances of neutralization were obtained, although, as will be seen, their value is somewhat doubtful.

Paramœcia will live for hours in strengths of sodium acetate varying at different times from $\frac{m}{20}$ to $\frac{m}{40}$, whereas hydrochloric acid is fatal in varying doses above 2 drops of $\frac{m}{500}$ HCl per 5 c.c. of H_2O . If at a given time I determined the dose of each which was just fatal within a certain period, I found that strengths of sodium acetate near the

least fatal, and others much below this, would so far inhibit the effects of hydrochloric acid that Paramœcia would live for hours in an amount of acid which alone would have coagulated them instantly. I cite as illustration an experiment that was made on Paramœcia from an acid culture. It was first found that sodium acetate killed within four hours in strengths above $\frac{m}{40}$, while anything stronger than 1 drop of $\frac{m}{500}$ HCl in 5 c.c. of H_2O coagulated them immediately. Three drops of $\frac{m}{500}$ HCl were added to 5 c.c. of each of the following strengths of acetate: $\frac{m}{50}$, $\frac{m}{60}$, $\frac{m}{80}$, $\frac{m}{100}$, $\frac{m}{150}$, $\frac{m}{200}$. The majority of individuals in $\frac{m}{50}$ to $\frac{m}{100}$ were living thirty-six hours later, when the last observations were made. The majority were dead within two hours in $\frac{m}{150}$, and only two or three remained in $\frac{m}{200}$. In the last two the strength of acetate was obviously not sufficient to counteract the effect of the acid.

In another experiment Paramœcia had been living for seventy hours in $\frac{m}{80}$, $\frac{m}{100}$, and $\frac{m}{200}$ solutions of sodium acetate. To each of these was added, a drop at a time, some of a solution of $\frac{m}{500}$ HCl, 2 drops of which killed Paramœcia from the culture immediately. At the end of an hour 23 drops had been added, and a few of the Paramœcia still lived. Just as striking were the results obtained with sodium citrate. It was used in strengths from $\frac{m}{500}$ to $\frac{m}{1000}$ against solutions containing from 2 to 7 drops of $\frac{m}{500}$ HCl. In all strengths used it had an inhibitory effect upon the action of the acid. In strong solutions, $\frac{m}{500}$ to $\frac{m}{1000}$, it was sufficient to retard for hours the effect of all strengths of hydrochloric acid, while in weaker solutions, $\frac{m}{800}$ – $\frac{m}{1000}$, only the weaker acid, 2 to 4 drops, was counteracted.

The reverse question, as to whether hydrochloric acid can be made to retard the injurious action of fatal doses of sodium acetate and sodium citrate, was less clearly answered. Strengths of hydrochloric acid which alone would not kill — subfatal doses — did not show any appreciable effect. Stronger solutions did, however, in the majority of cases, delay the action of the acetate and citrate, although in no instance was any very decided result obtained.

Combinations of calcium chloride with sodium acetate gave the following result: the action of fatal amounts of calcium chloride was not counteracted by any strengths of sodium acetate, fatal or subfatal, but solutions of calcium chloride in strengths considerably below fatal exerted a marked retarding influence upon the action of fatal sodium acetate.

In these experiments it is clear that the effect of hydrochloric acid

can be neutralized much more readily than that of calcium chloride. This suggests the idea that in the former case some acetic and citric acids may be formed in the mixture. As these are much less readily dissociated than hydrochloric acid, they would have a proportionately less injurious effect upon the organism. The "neutralization" may therefore be one of reagents rather than of effects.

One further series of phenomena seems to me suggestive. This was often observed where subfatal doses of antagonistic substances were mixed, as, for example, sodium acetate and hydrochloric acid, cane-sugar and potassium hydrate, etc. In many cases such mixtures proved fatal. I cite a couple of instances from my experiments.

1. 5 c.c. $\frac{m}{20}$ Na acetate. Paramœcia living after 3 hours.
5 c.c. H_2O + 1 drop $\frac{m}{100}$ HCl. Paramœcia living after 3 hours.
5 c.c. $\frac{m}{20}$ Na acetate + 1 drop $\frac{m}{100}$ HCl. Paramœcia dead in 1 hour.
2. 25 c.c. $\frac{m}{8}$ cane-sugar. Paramœcia living after 24 hours.
5 c.c. H_2O + 5 drops $\frac{m}{50}$ KOH. Paramœcia living after 24 hours.
5 c.c. $\frac{m}{8}$ cane-sugar + 5 drops $\frac{m}{50}$ KOH. Majority dead in 40 minutes.

The first explanation that suggests itself is that by the method used there is, in 5 c.c. of each mixture, a much greater quantity of total dissolved substance than is present in either control. For this reason the osmotic pressure is increased, and more rapid death is to be expected. But, as has been seen, Paramœcia are sensitive only to great changes in osmotic pressure, far above those involved in the mixtures used. And, moreover, observation of Paramœcia dying in such mixtures showed that the effect was not shrinkage due to loss of water, but rather increased swelling and more rapid disintegration. Recalling the fact that all coagulating agents studied showed a secondary action tending to produce liquefaction, it seems probable that we are here dealing with the addition of secondary to primary liquefying influences.

Nor is this all, for I observed that in the mixture of cane-sugar and sodium citrate the vesicles which formed differed entirely in character from those formed in solutions of either agent alone. Instead of being large, slightly refractive, and soluble in water, they were small, highly refractive, and floated in the water like oil globules. Obviously, here, the effects are not even purely additive, but one agent so modifies either the other agent, the protoplasm itself, or the cell membrane, that a new effect is produced.

CONCLUSION.

It is evident from the results described that in studying the reactions of Protozoa according to the methods usually employed, we are dealing with a number of factors in which the proportion of unknown so far exceeds the known that a proper interpretation of results is almost impossible. Even so simple a phenomenon as the indefinite life of *Paramœcia* in distilled water cannot be explained. Is there, according to simple physical laws, an outward diffusion of the ions of the protoplasm until equilibrium is established? If so, can the protoplasm be kept active by the stimulating effect of H and OH ions from the water?¹ If not, are the salts held in loose chemical combination with the proteid molecule,² or is there a change in the permeability of the cell membrane such that diffusion is prevented?³ That there is some change is indicated by the fact that the organisms become more sensitive after exposure to water.

Again, no two cultures are ever chemically the same, nor does the same culture remain constant for long periods of time. Much stress has previously been laid upon variations in acidity or alkalinity, but other substances must also exert an influence, if we assume the protoplasmic membrane to be permeable to substances in solution.

Does the stage in the life history of *Paramœcia* affect their sensitiveness or the nature of their response? In an experiment with sodium acetate I used *Paramœcia* of which a large number were conjugating. Here I noted that the bottom of the dish was soon covered with paired individuals, while the others lived long after. There may even prove to be rhythmical changes in sensitiveness like those described by Lyon⁴ for cleaving eggs, and Scott⁵ for unfertilized eggs. Something of this nature is indicated by the fact that *Paramœcia* from the same culture vary in sensitiveness from day to day.

Different species of *Paramœcia* from the same culture vary in their response. At one time I used a culture which contained individuals of two species—one large, one small. They were exposed (a) to calcium chloride, and (b) to sodium sulphate. In (a) the large

¹ MATHEWS: This journal, 1904, x, p. 290.

² LOEB: *Ibid.*, 1900, iii, p. 327.

³ HOWELL: *Ibid.*, 1901, vi, p. 181.

⁴ LYON: *Ibid.*, 1902, vii, p. 56; 1904, xi, p. 52.

⁵ SCOTT: Biological Bulletin, 1903, v, p. 35.

Paramœcia were much more sensitive; they became swollen at the anterior end and soon burst. Later the small ones died, but these were shrunken and rigid. In (b) the smaller ones became swollen, vesicles appeared, and they soon died, whereas the large ones lived for some time.

Finally we are, I think, forced to the conclusion that the terms "coagulation" and "liquefaction" at present serve largely to cover our ignorance, for they express only in the vaguest way the processes that take place in living organisms in response to different stimuli. The retarding influence of lowered temperature, the secondary effects which take place in certain reagents, and the appearance of new phenomena when mixtures of subfatal solutions are used — in short, all the discrepancies met in an effort to neutralize one effect by another — point to the occurrence in the protoplasm of extensive changes, physical and chemical, which it is impossible, in the present state of our knowledge, to analyze fully. The first step toward a clearing of the haze that envelops the subject will be found, I believe, when an effort is made to unify the conditions under which different investigators are working.

I desire to express my appreciation of the kindness shown me by Dr. E. P. Lyon, under whose direction this work was done, and to whom I am greatly indebted for suggestion and criticism.

SUMMARY.

Paramœcia will live indefinitely in perfectly pure water. Immersion in water causes a variable degree of increase in the sensitiveness of the organism toward many chemical reagents.

The constitution of the original culture — apart from its content of acid or alkali — and the time when an experiment is performed, are both factors in determining the reaction of Paramœcia to chemical and physical stimuli. The reaction of Paramœcia reared in acid, neutral, and alkaline cultures varies but little qualitatively.

Paramœcia become readily habituated to solutions in strengths which are not soon fatal.

Low temperature causes loss of water and condensation of the protoplasm. It also retards the action of the following substances upon Paramœcia; sodium hydrate, sodium acetate, sodium citrate, sodium chloride, sodium sulphate, hydrochloric acid, calcium chloride, cane-sugar, urea.

Of the above substances, the effect of those with predominant anions is to liquefy the protoplasm, but the behavior of *Paramœcia* varies greatly according to the liquefying agent used.

The primary effect of solutions containing predominant kations is to cause condensation of the protoplasm, but secondary effects may follow which tend toward liquefaction.

Strongly osmotic solutions of cane-sugar and urea also have two effects; the first leads to loss of water and condensation, the second to swelling and disintegration of the protoplasm.

By combining solutions whose effects are antagonistic, a variable amount of retardation can be obtained. Such solutions are cane-sugar and potassium hydrate, cane-sugar and sodium citrate, calcium chloride and sodium acetate, and mixtures of hydrochloric acid with sodium acetate and sodium citrate. Retardation is most striking in the last instance, but may here be due to dissociation changes in the solutions themselves.

In urea¹ the secondary influence so predominates that when it is mixed with potassium hydrate or sodium citrate an additive effect is produced, while partial neutralization is obtained when hydrochloric acid is used.

A mixture of subfatal doses of apparently antagonistic substances may prove more fatal in its effect than is either substance alone.

Complete neutralization of the effects of antagonistic substances is never obtained.

¹ See note 2, p. 228.

THE FATE OF STRYCHNINE IN THE INTESTINE OF THE RABBIT.

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W. SALANT,¹ in 1902, published a statement to the effect that the cæcum or colon of the rabbit contains a substance capable of destroying strychnine. If a milligram of strychnine be injected into that portion of the intestine, it disappears, and no known test will discover it.

At that time Professor Torald Sollmann suggested to me that this phenomenon was very important, and it should receive further investigation. I accordingly began the experiments reported in this paper.

Since then Salant² has partially confirmed his former work; but since his results and mine do not agree in one or two minor particulars, I have concluded to publish my experiments, especially in view of the fact that the intestinal contents undoubtedly render the detection of strychnine difficult.

The first attempt to estimate quantitatively the strychnine extracted from the mixture of 0.01 gram with the contents of the rabbit's cæcum and colon proved so unsatisfactory that an effort was made to determine the amount necessary to produce convulsions when injected into the cæcum, in order to compare the results with those obtained when the strychnine was given by the mouth. The rabbits were lightly anesthetized with ether, the intestines exposed, and the animals allowed to come from under the influence of ether before the injections of strychnine into the colon were made.

Many disturbing factors are involved in this procedure. The rate of absorption will depend much upon the contents of the intestine. The tetanic dose of strychnine varies enormously when anesthetics are employed, thirty times the normally convulsive dose

¹ SALANT, W.: *Centralblatt für innere Medicin*, 1902, p. 1089.

² SALANT, W.: *Journal of medical research*, 1904, p. 41.

having repeatedly failed in some of our laboratory experiments after urethane and ether had been employed. The results were so variable as to prove wholly unsatisfactory. The following method was therefore adopted:

Method. — One milligram of strychnine (1 c.c. of $\frac{1}{1000}$ solution) was injected into the colon of an anæsthetized rabbit, and the colon and cæcum were at once removed to a vessel in which the intestine and its contents, thoroughly mixed, were allowed to stand at room temperature for an hour in order to permit the destructive action of the unknown substance for a period at least equalling that during which strychnine might be expected to remain in the intestine of the living animal. The mixture was then extracted with 500 c.c. of water acidulated with tartaric acid. On boiling the mixture and allowing it to stand, the coagulated proteid carries suspended matter down, permitting of more ready filtration. The extraction was repeated with about 500 c.c. of water acidulated as before; this was filtered, and the mixed filtrates evaporated upon a boiling-water bath to a syrupy consistence. The residue was twice extracted with about 100 c.c. of alcohol, filtered, and the mixed filtrates evaporated upon a water bath almost to dryness. The residue was taken up with a little very dilute sulphuric acid, washed with ether, made alkaline with ammonia, and extracted several times with chloroform and ether. After distilling off the chloroform and ether, further purification was effected by repeating the above process several times, the chloroform and ether extraction being several times repeated in each instance. The final residue after distilling the chloroform-ether solution was dissolved in a little water slightly acidulated with hydrochloric acid, the excess of acid being neutralized with ammonia water and the whole evaporated to about 2 c.c. About half of this solution was injected into a small frog of approximately fifteen grams weight. Typical strychnine convulsions occurred in three minutes. The next morning the frog was normal. Since about an eighth of a milligram of strychnine would be fatal to a frog of that size, it is evident that not all of the strychnine was recovered. The following experiments were performed with this method:

Experiment 1. — Ten milligrams of strychnine sulphate were placed in the cæcum of a recently killed rabbit. The contents were extracted after the manner detailed above. After purification three-fourths of the resulting extract was injected into a frog weighing thirty grams; depression without hyperexcitability followed for twenty minutes or more, but typical strych-

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nine convulsions occurred within fifty minutes. The frog was normal next morning. The remainder of the extract acidulated with sulphuric acid gave no precipitate with Mayer's reagent. Potassium bichromate and sulphuric acid were also negative.

Experiment 2. — The contents of the large intestine of a small rabbit were divided into two portions, the first portion was boiled, the second was not; to each portion were added two milligrams of strychnine sulphate, and each was separately extracted as detailed above and the solution of the purified extract made up to 5 c.c. One cubic centimetre of the first solution injected into a frog rendered it hyperexcitable, and after an hour tetanus could be induced by jarring. The frog appeared normal in an hour and a half after the injection. One cubic centimetre of the second extract was injected into a frog. It became tetanic in half an hour, and appeared to be dead in two hours and a half.

Experiment 3. — A rabbit weighing 1430 gms. was lightly anaesthetized with ether, the intestine exposed and the ether withdrawn. Ten milligrams of strychnine sulphate were injected into the large intestine. No effect being perceived in thirty minutes, the dose was repeated; twenty minutes later a severe convulsion occurred; one hour after the first injection the rabbit was killed and the contents of the intestine removed. This was extracted in the manner described, the resulting extract being made up to 10 c.c. Although this solution had a persistently bitter taste, which was not true in the other cases, half a cubic centimetre injected into a frog induced convulsions only after three-quarters of an hour.

*Experiment 4.*¹ — This is the experiment detailed in the text, one milligram of strychnine sulphate being injected into the colon of a rabbit and extracted.

Experiment 5. — One cubic centimetre of distilled water was injected into the colon of a rabbit, and the contents of the colon and caecum extracted as in Experiment 4, this serving as a control of that experiment. Half of the resulting extract was injected into the same frog used in Experiment 4 about a week previously. After a time the frog became comatose, but no convulsions occurred.

The difficulty of recovering strychnine quantitatively was shown by the author, in 1902, in a series of experiments² undertaken to learn whether the physiological test upon frogs might be used

¹ The extractions in Experiments 4 and 5 were done under my direction by Mr. J. WALLACH.

² HATCHER, ROBERT A.: American journal of pharmacy, 1902, lxxiv, p. 283.

quantitatively. In these experiments from 0.6 milligram to 10 milligrams was injected into living rabbits and guinea pigs, and extracted by a process similar to that mentioned above. In one case only did the physiological test indicate that nearly all the strychnine had been recovered. I quote from these experiments: "Calculating the dose of strychnine sulphate extracted from the tissues from the amount injected, ten milligrams, and supposing none to have been lost or destroyed, the extract, after repeated purification, proved fatal to a frog in the dose of 0.0065 milligram per gram of frog. An equal dose suspended in thick mucilage of acacia was survived. This was much above the dose which is fatal in the absence of colloid."

When the smallness of the amount used in the latest experiments, one milligram, is considered, it is not remarkable that more was not recovered. This small dose did not permit of chemical tests, but reference to Experiment 1, given above, will show that, when larger amounts were injected into the rabbit's intestines and extracted by similar methods, the chemical test proved negative, probably owing to organic combination.

It may therefore be concluded with certainty that the contents of the rabbit's cæcum and colon do not completely destroy strychnine under the conditions of these experiments, and it appears somewhat doubtful if any actual destruction occurs under any condition. The amount of strychnine extracted seems to bear little relation to the amount injected. With very small quantities, the difference apparently depends upon differences in manipulation too slight to be controlled. In one instance, after mixing two milligrams with the contents of the rabbit's intestine, one-fifth of the extract was fatal to a frog, whereas in another, three-fourths of the extract obtained from an injection of ten milligrams, or theoretically about nineteen times the former dose, failed to cause death.

The use of heat in the extraction does not seem to be the main disturbing factor, as Salant suggests, since heat was successfully employed with such a small quantity as one milligram.



DIFFERENCES IN ELECTRICAL POTENTIAL IN DEVELOPING EGGS.

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INTRODUCTION.

WHILE studying the embryology of some Scyphomedusæ¹ I observed that the development of the eggs in sea-water which had become concentrated by evaporation differed in certain respects from that seen in an aquarium constantly supplied with running water. In some of the eggs nuclear division had taken place, but the cytoplasmic division had been prevented, while other eggs had encysted, and continued their development to certain stages in the encysting capsule. Loeb² had previously observed in other eggs that during the segmentation period only the nuclei divided, and many investigators have since that time seen this fact demonstrated in the eggs of Echinoderms and worms, which had been placed in strong solutions of electrolytes. Loeb's³ explanation for this phenomenon is, that the segmentation of the protoplasm is the effect of a stimulus which the nucleus applies to the protoplasm and which makes the protoplasm close around the nucleus. On the other hand, if we put an egg in sea-water whose concentration has been raised by certain salts, the protoplasm loses water and the loss of water brings about a loss of irritability, and consequently the cytoplasm fails to segment. There is therefore a certain concentration at which the nucleus is still able to divide, while the protoplasm loses its ability to respond to the stimuli emanating from the nucleus.

Moreover the phenomena of cell division are, according to Loeb,⁴ Bütschli,⁵ and Quincke, dependent upon protoplasmic streaming.

¹ HYDE, I. H.: *Zeitschrift für wissenschaftliche Zoologie*, 1894, lviii, p. 532.

² LOEB, J.: *Journal of morphology*, 1892, p. 253.

³ LOEB, J.: *This journal*, 1900, iii, p. 435.

⁴ LOEB, J.: *This journal*, 1902, vi, p. 432.

⁵ BÜTSCHLI, O.: *Untersuchungen über mikroskopische Schäume und das Protoplasm*, 1892.

This requires as Quincke has shown, a definite degree of viscosity. If the viscosity is too great or too small, no protoplasmic streaming is possible. Since the viscosity is altered by the presence of electrolytes, the streaming of the protoplasm must also be influenced by the presence of these.

We learn from D'Arsonval¹ that changes in surface tension also produce protoplasmic streaming, and he shows that on the basis of Lippmann's observations electrical charges must lead to changes in surface tension. Loeb also believes that there is a possibility that part of the chemical energy in the development of the egg is converted into some form of electrical energy, that the ions formed in metabolism play a rôle in the dynamics of life-phenomena, and that these ions or electrical charges may be responsible for such physical manifestations as cell development and segmentation.

Besides these, other investigators who have studied the mechanics of mitosis agree that a decrease in surface tension over a definite area of the egg, and a flowing state of the protoplasm are two of the factors upon which segmentation depends. We may assume that these conditions are in turn produced by certain definite physico-chemical changes occurring among the nuclear and cytoplasmic elements of the egg, and also between the egg itself and its surrounding medium. Of these changes, the most efficient are the actions of the ions through their electrical charges, the enzymes, and a certain amount of water. It would seem therefore that in the concentrated sea-water, the absorption of water from the egg's contents instituted a condition in the egg which interfered with the normal actions of its ions, and probably also its enzymes, so that segmentation of the cytoplasm did not proceed.

I believe that physiological or metabolic changes dependent upon certain definite physical interactions of electrolytes and colloids must occur throughout the life-history of the developing egg, and are accompanied by differences of electrical potential, which, if demonstrable, would give information of certain changes progressing in the egg throughout its ontogeny. The differences of potential might be employed, for instance, as a measure to ascertain the time in the development of the egg when the phases of heightened and lowered activities occur, the regions in which they take place, the relation of the electrical organization in the egg to that in the embryo and

¹ D'ARSONVAL: *Archives de Physiologie*, 1889, p. 460.

adult, as well as the effect produced by various external influences or solutions upon the different phases and the time of their occurrence. I determined, therefore, to ascertain if a difference of electrical potential in the egg could be detected. Several kinds of eggs were tested at the Naples Zoölogical station, with a very sensitive capillary electrometer, constructed especially for this purpose. I had the satisfaction of learning that an electrical difference of potential could be detected. My time was however occupied with other work, so that the study on electrical polarity was postponed.

During the summer of 1902, while at Wood's Hole, I resumed the investigation begun six years previously, but did not publish the results at once, because I decided to investigate the egg of the Toad-fish, which matures early in June. Since I first began this investigation many important contributions bearing upon the physical and chemical changes in protoplasm, especially regarding its colloids and ions have enriched this field of physiology. These achievements have not only strengthened the belief in the chemical and electrical phenomena accompanying development but have offered results with the aid of which the existence of these phenomena can be better understood and explained.

I now endeavored to ascertain whether in maturing and fertilized eggs of different animals the differences of electrical potential were related to known embryological stages, and whether there existed an electrical polarity related to the astral radiations, spindle fibres, and polarity of the egg which Driesch¹ maintains exists in every cell, and upon which segmentation and organization depend. I desired also to determine whether, in fertilized eggs, rhythms of differences of electrical potential might be discovered which would coincide with those special rhythms discovered by Mrs. Andrews² in starfish and *Echinus* eggs. In the eggs of these forms Mrs. Andrews found that the time required for staining or fixing reagents to act varied rhythmically, and that these rhythms are sympathetic with rhythms of resistances to pressure. Then, too, I wished to learn whether rhythms of differences of electrical potential are related to the periods of resistance, to lack of oxygen, presence of potassium cyanide, and production of carbon dioxide which Lyon³ found in *Arbacia* eggs. These periods may be related to those of susceptibility to mechanical agitation

¹ DRIESCH, H.: *Archiv für Entwicklungsmechanik*, 1897, iv, p. 79.

² ANDREWS, G. F.: *Journal of morphology*; supplement, 1897, xii, pp. 30-57.

³ LYON, E. P.: *This journal*, 1902, vii, p. 56; 1904, xi, p. 58.

found by Scott¹ in unfertilized eggs of *Amphitrite*, and to the stages in the egg of the starfish highly susceptible to artificial fertilization, which Delâge² believes occur, at a time between the breaking down of the germinal vesicle and the appearance of the first polar body. Another question which might be worth considering is, whether the electrical potential is increased or decreased by changing the environment of the egg, either by changes in temperature or ions in the surrounding medium, and whether such changes in difference of potential bear a definite relation to the susceptibility to artificial parthenogenesis.

METHODS.

The difference of potential existing between two parts of the egg was determined with a D'Arsonval galvanometer, as well as with the most delicate capillary electrometers of the modified Lippmann, Porter,³ and Lyon⁴ types. Purified mercury, chemically pure sulphuric acid, and glass parts that were thoroughly cleansed, were used in the construction of the electrometer.

The movements of the meniscus in the capillary were measured with an ocular micrometer scale. Each division indicated approximately 0.00001 volt. The nonpolarizable electrodes were composed of zinc, zinc sulphate, clay, and tiny brushes. The clay and brushes were moistened with $\frac{m}{8}$ sodium chloride solution, isotonic with the fluid contents of the turtle and frog's body or egg, or an $m \frac{5}{8}$ sodium chloride solution, when working with fishes' eggs. The electrodes were tested before and after each reading, in order to keep them isoelectric for a considerable time. Each part composing the electrometer and connected parts received the most careful attention. The apparatus stood on a firm stone foundation, so that all external vibrations were eliminated, and the temperature of the room was constantly taken into consideration. The eggs and brush electrodes were kept at practically the same degree of moisture throughout the observations.

¹ SCOTT, J. W.: Biological bulletin, 1903, v, p. 35.

² DELÂGE, Y.: Archives de zoologie expérimentale et générale 1901, ix, p. 88.

³ Used in the physiological laboratory of the Harvard Medical School.

⁴ Used in the physiological laboratory of the University of Chicago.

MATERIAL.

Eggs from different types of animals were investigated. Those of invertebrates and of most fishes were too small to be advantageously employed. Nevertheless, besides some unsatisfactory results with eggs from the toadfish, a series of observations were obtained with greatest difficulty and care, from the eggs taken from *Fundulus*. A few observations were made on toads' eggs and more on eggs from turtles.

The records of a few experiments conducted on an uncertain species of toad's eggs, several hours after fertilization, indicated a difference of electrical potential of a mean of 0.00002 volt, between the poles of an axis, at an angle to an axis directly through the pigmented and unpigmented regions of the egg. In tadpoles from eight to ten days old the difference of potential was about 0.0002 volt, and in a direction from the tail to the head of the embryo. If the first cleavage plane corresponds to the median plane of the toad's body, the direction of the current in the egg and embryo is the same in reference to the first cleavage. The results obtained from the toad and toadfish were too meagre to be of value.

DIFFERENCES IN ELECTRICAL POTENTIAL IN THE TURTLE'S
EGG. (*CHRYSAMYS PICTA*.)

The egg was taken after decapitation from the ovary or oviduct. Adhering fluid or connective tissue was carefully removed, and the egg placed on filter paper moistened with $\frac{m}{8}$ salt solution. The egg was touched only with camel's hair brushes, or delicate paraffine-covered egg lifters. Eggs were studied at different stages of their maturity. They ranged from five to twenty millimetres in diameter. They are somewhat ellipsoidal in form. The germinal disc, with one or more brownish micropyles, is recognized at one pole by its lighter yellow color. The eggs are fertilized in the ovary, and the segmentation nucleus lies directly under the germinal disc during maturation, but after fertilization it is further from the surface. The egg is surrounded, in the late segmentation stages, by a closely adhering glary mucilaginous membrane which cannot be removed without injury to the egg. Therefore only the eggs in the maturation and first segmentation stages proved available.

A. The first object was to determine if a difference of potential existed between the animal and vegetative poles, and if so, the direction of the current in the egg. For this purpose the egg was placed with its respective poles in contact with the brushes of the non-polarizable electrodes. As usual, the scale reading was first obtained with both brushes in contact, to show whether the elec-

TABLE I.

(A).—SHOWING THE DIFFERENCE OF POTENTIAL BETWEEN THE BLASTODISC AND VEGETATIVE POLE OF THE MATURE TURTLE'S EGG.

Experi- ment.	Diameter in centimetres	Scale readings in 0.00003 Volt.	Direction of the current in the egg from the blastodisc toward the vegetative pole.	Eggs reversed.
9	0.5	1	" "	Current reversed.
8	0.5	2	" "	" "
27	1.0	2	" "	" "
4	1.5	3	" "	" "
20	1.5	5	" "	" "
15	1.6	4	" "	" "
31	1.8	6	" "	" "
7	1.2	3	From the vegetative pole toward the blastodisc.	" "
25	1.6	9	" "	" "
(B).—MEAN RESULTS FOLLOWING PUNCTURE TO THE VEGETATIVE POLE OF THE TURTLE'S EGG.				
32	1.5	3	Direction of the current in the egg from the blastodisc to the vege- tative pole.	Eggs uninjured.
33	1.5	5	Vegetative pole to the blastodisc.	Eggs injured and not reversed.

trodes were isoelectric. When several readings had been secured with the egg in one position, the egg was reversed, and the extent and direction of the movements of the meniscus of the mercury, or of the scale readings, were compared with those of the first readings.

Table I is a record of a few of many observations of the differences of potential existing between the animal and vegetative poles of the

turtle's egg. It shows that the more mature eggs have a greater difference of potential than have the smaller less developed ones. Though as a rule, the difference of potential varies within small limits between those of the same size, nevertheless, the least current is found in the smallest eggs. With but few exceptions the direction of the current in the egg was from the animal toward the vegetative pole. The records indicate that as maturation of the egg progresses, metabolism increases, and that its seat of action is greater at the animal pole.

B. From Experiments 7 and 25, Table I, it will be observed that the direction of the current in these eggs is the reverse of that observed in the other eggs. This indicates that at the time the read-

TABLE II.
DIFFERENCES OF POTENTIAL BETWEEN THE POLES OF THE MAIN AXIS OF THE
BLASTODISC IN THE TURTLE'S EGG.

Experiments.	Egg diameter in cms.	Scale reading in 0.00003 volt.
28	1.0	2
27	1.3	4
25	1.5	4
21	1.6	7
20	1.6	8
29	1.8	7

ing was taken, there was a greater difference of potential at the vegetative pole. To ascertain whether this may be due to injury, several eggs were slightly punctured at the vegetative pole. The mean result obtained from these eggs, as stated in B, Table I, shows not only that the current was reversed after injury to this pole, but that it was in excess over that existing in the egg before it was punctured. The current of injury which was produced may be the result of factors similar to those that cause the demarcation current in nerve-muscle preparations.

C. It was next of interest to determine if differences of electrical potential existed between the poles of the main axis in the blastodisc. If the egg is placed on a glass stand so that the blastodisc is upper-

most, and the tiny non-polarizable electrodes are placed at the same level at different, opposite points of the disc, it is found that there are two such points that exhibit the greatest difference of potential. These poles bear a definite relation to the long axis of the egg, and may correspond to the future head and tail end of the body. They may be considered the poles of the main axis. Accordingly, an axis midway and perpendicular to the main axis, would point to the right and left sides of the body.

A few results of many experiments conducted for the purpose of ascertaining whether there is a fixed polarity in the blastodisc, are tabulated in Table II. It was learned that the same strength of current or difference of potential was registered, but reversed in direction, when the electrodes were placed at reversed poles of the main axis

TABLE III.

MEAN RESULTS OF SEVERAL EXPERIMENTS TO DETERMINE THE DIFFERENCE OF POTENTIAL EXISTING AT DIFFERENT LEVELS BELOW THE BLASTODISC OF THE MATURATING TURTLE'S EGG.

Experiments.	Size in cms.	Axial distance below the blastodisc.	Scale reading in 0.00003 volt.
23	1.5	One-fourth	5
23	1.5	Half-way	0
23	1.5	Three-fourths	3
8	0.5	Half-way	0

of the blastodisc, or if the egg was reversed. This proved that there is a fixed polarity in the blastodisc of the egg, and that there is a determinable constant current flowing in a definite direction in it. Whether this current is from the tail toward the head end of the adult, or *vice versa*, can be proved only by comparing the current in the axis in the animal area throughout the different phases of development. When the electrodes are placed at the poles of an axis perpendicular to the main one, as a rule no difference of potential is detectable, though in a few instances a slight, fixed current was observed.

D. Differences of electrical potential between the poles of an axis at different distances below the blastodisc of the maturing turtle's egg were next investigated. When the electrodes are placed

at the poles of an axis, parallel to the blastodisc, and below it, that is, perpendicular to an axis extending through the animal and vegetative poles, it is proved that the difference of potential decreases as the electrodes are shifted further away from the blastodisc area. Half-way down, there is one diameter at which the meniscus of the mercury registered no difference of potential between the poles. In some eggs the difference of potential was zero half-way down; but three-fourths down it was above zero, as tabulated in Table III. Possibly this was because the blastodisc is exequatric, and the electrodes were placed at unequal distances from the pole of the highest potential.

The experiments on the maturing turtle's egg proved the presence of a difference of electrical potential between the blastodisc and the opposite pole of the egg. This difference increases with the development of the egg, and is probably the result of chemical or physico-chemical activity in the blastodisc area predominating over that of the vegetative pole. From the fact that a difference of potential exists between the poles of the blastodisc area, producing a current in a definite direction as regards both the blastodisc and the egg as a whole, it is evident that a fixed polarity exists in the blastodisc. It is possibly due to this polarity that a difference of potential is detectable at different levels below the blastodisc pole, and perpendicular to the main axis of the egg.

II. CHANGES IN ELECTRICAL POTENTIAL IN THE EGG OF FUNDULUS AFTER FERTILIZATION.

Eggs from *Fundulus* were obtained by stripping the fish. The eggs were fertilized in sea-water, in which they were kept under observation throughout the experiment.

The appearance of external changes, as well as the time of their occurrence, were noted; it was therefore possible to ascertain whether development between the brush electrodes was at all times proceeding normally. The first cleavage appeared in about one and one-half hours. The second about one hour later, and the third about forty-five minutes after the second, depending upon the temperature of the water. The eggs are from two to two and one-half millimetres in diameter, and the germinal disc is easily recognized by its whitish color. The brush electrodes and the clay were moistened with *m* sodium chloride solution isotonic with that of sea-water, and th

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TABLE III.

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Experiments.	Size in cms.	Axial distance below the blastodisc.	Scale reading in 0.00003 volt.
23	1.5	One-fourth	5
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eggs were washed in $m \frac{5}{8}$ salt solution, before being placed between the brushes. Each division of the scale in the ocular micrometer indicated 0.00001 volt; the resistance of the non-polarizable electrodes was determined to be 10000 ohms.

It required many patient efforts before a reading of the movements of the mercury meniscus could be recorded. Only four experiments

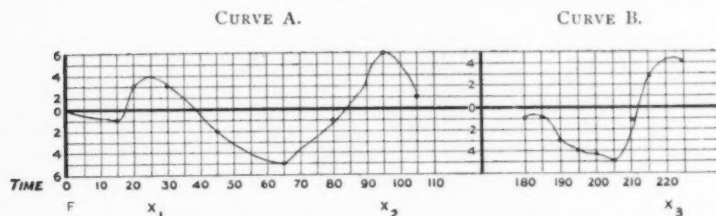
TABLE IV.
DIFFERENCES OF ELECTRICAL POTENTIAL BETWEEN THE ANIMAL AND
* VEGETATIVE POLES IN FERTILIZED EGGS OF FUNDULUS.

A.			B.		
Time in minutes after fertilization.	Scale division in 0.00001 volt.	Observation on the egg.	Time in minutes after fertilization.	Scale division in 0.00001 volt.	Observation on the egg.
15	-1 *	Segmentation. Nucleus formed.	180	-1	4-cell stage.
20	+2 †		185	-1	
30	+2		190	-3	
45	-2		195	-3	
55	-3		200	-4	
60	-4		205	-5	
65	-5		210	-1	
75	-2		215	+3	
80	-1	First segmentation. Furrow.	225	+4	8-cell stage.
90	+4				
95	+5				
105	+1				

* + means that the current in the egg is from the blastodisc toward the opposite pole.
† - means that the current is from the opposite pole to the blastodisc.

were satisfactory, of these, two are shown in Table IV. The first record (A) was secured fifteen minutes after the eggs were fertilized. The egg under observation and the electrodes were placed in a moist chamber, that is, in a glass vessel in which was placed moist filter-paper, so that evaporation from brushes and eggs was avoided. The

difference of potential of the egg existing between the animal and vegetative poles was obtained at consecutive intervals of time by placing the egg in the electrometer circuit for a moment, and observing the extent and direction of the movements of the meniscus of the mercury. The egg was under observation for one hour and thirty minutes. The first segmentation furrow had appeared after ninety minutes. The plus and minus sign before the number of scale divisions indicate the direction of the current in the egg. The plus is to express the direction of the current from the blastodisc to the opposite pole, the minus the current in the egg, in the reverse direction, that is, from the vegetative pole toward the blastodisc. It is seen that the difference of potential is not constant, and that it shifts, during the interval from the time of fertilization until the appearance



CURVE A illustrates Table IV. It shows the changes in differences of electrical potential in the egg of *Fundulus* from fertilization to the first segmentation furrow. CURVE B illustrates the changes in the differences of electrical potential after the second to the third segmentation furrow. At F, fertilization of the egg took place; X₁, formation of the segmentation nucleus; X₂, first segmentation furrow appeared; X₃, third segmentation furrow appeared. The abscissa indicates time in minutes; the ordinate, the scale readings in direction and strength of electromotive force.

of the segmentation furrow, from one pole to the other. For instance, fifteen minutes after fertilization, the current in the egg was from the vegetative toward the animal pole, and the difference of potential was only one scale division, or about 0.00001 volt; since the scale divisions were so close, it was difficult to estimate whether it was the whole or only a fraction of the division. Five minutes later a great change was indicated, the current was now from the blastodisc and the electromotive force two scale divisions. The next reading was taken ten minutes later, and showed no change in electromotive force and direction of the current. In the long interval of ten minutes, however, judging from the observations made on other eggs, some changes must have occurred. Fifteen minutes later the current was reversed, and during the succeeding twenty minutes it had

gradually increased to its greatest strength, while during the following fifteen minutes it fell to its least. By the time the next record was secured ten minutes had passed, and in that time the first segmentation furrow had appeared. The current changed its direction, and in five minutes again gained its greatest electromotive force, only to again lower it to its least amount in the succeeding ten minutes.

The second record (B), Table IV, shows practically the same relation of increase and decrease of potential after cleavage had begun as was found in the other records, following a certain interval of the time after fertilization.

A COMPARISON OF MY OBSERVATIONS WITH THOSE OBTAINED
BY OTHERS.

The records of the differences of electrical potential obtained from the fertilized eggs of *Fundulus* would indicate that from the time of fertilization until the appearance of the first cleavage, and from that time onward, pronounced physiological activities took place accompanied by electromotive changes. Judging from the direction and extent of these changes, several distinct phases are discernible. The first begins with fertilization and extends over a period of about fifteen minutes, after which a change in the direction of the current is manifested. The second phase continues about fifteen minutes, during which time the current is again reversed. The third phase continues about thirty-five minutes. It is characterized by a gradual increase in difference of electrical potential. The fourth lasts about fifteen minutes, during which time the difference of potential falls from its greatest to its least value. The fifth is illustrated by a change in the direction of the current and a rise in the difference of electrical potential, and during this phase the first segmentation furrow appears. The sixth is noted by a gradual fall in potential.

About twenty minutes after fertilization, near the time when the first segmentation nucleus is formed, and again at the time of the appearance of the segmentation furrow, the current in the egg has the same direction, that is, the seat of greatest potential, or physico-chemical change is in the same region of the egg. The direction of the current is from the blastodisc toward the vegetative pole, before the eggs are fertilized. It changes its direction after fertilization. From the observations noted in Table IV, and curve (A), it is evi-

dent, that there are rhythms in regard to the changes in the direction of the current. After fertilization there is a negative current, that is, one whose direction is towards the blastodisc; it may be due to changes in chemical reactions that affect physical changes. Then a positive current sets in, arising from the blastodisc and followed by a current first negative and then positive. Moreover these rhythms are characterized by an increase and then a fall of potential.

These phases as well as their causes may be variously interpreted by embryologists, microchemists, and physico-chemists. From the embryological standpoint, the different phases of electrical potential might be compared with certain phases in the egg during the interval between fertilization and cleavage. The first, for instance, might be compared with the period occupied by the entrance of the spermatozoön and changes instituted thereby in the egg; the second, with the time of fusion of the male and female pronuclei and the formation of the segmentation nucleus; the third, with the prophase; the fourth, with the metaphase; the fifth, with the anaphase; and the sixth, with the telophase.

The curves of electrical potential may furthermore bear a definite relation to rhythms of viscosity and pigment wandering; to phases of resistance of the mass to pressure, and to the special chemical reactions of the astral rays, which Mrs. Andrews¹ observed in starfish and sea-urchins' eggs during maturation and segmentation. The facts may also be brought into relation with the staining reactions of chromatin as observed by Heidenhain² and Rückert³ who concluded that the proportion of nucleic acid and albumin varies with periodical changes in the nucleus, and that these are related to changes in the function of the nucleus and to definite mitotic phases.

Especially may certain phases of these rhythms of differences of potential be compared to those of resistance to lack of oxygen, or resistance to potassium-cyanide, or to those rhythms of production of carbon-dioxide during cleavage that were found by Lyon⁴ to exist in the Echinoderm egg. Lyon found that the Echinoderm egg was more

¹ ANDREWS, Mrs. G. F.: *Journal of morphology*; supplement, 1897, xii, pp. 30-57.

² HEIDENHAIN: *Archiv für mikroskopische Anatomie*, 1894, xliii, p. 423.

³ RÜCKERT: *Anatomischer Anzeiger*, 1892, vii, p. 107.

⁴ LYON, E. P.: *This journal*, 1902, vii, p. 56.

susceptible to lack of oxygen, or less resistant to potassium cyanide about ten to fifteen minutes after fertilization, and again about the time of division. From the most susceptible stage, there is a decrease in susceptibility to lack of oxygen, or increase in resistance to potassium-cyanide up to the time of cleavage. Similar variations had been found by him in the interval between the second and third cleavage. The first cleavage appears in *Arbacia* about fifty to sixty minutes after fertilization, so the rhythms of susceptibility are about thirty-five to forty-five minutes apart. The inference seems to be, that between the susceptible periods the nuclear membrane is dissolved and the chromatin more widely distributed in the egg-substance, while after each segmentation the nuclear membrane keeps the chromatin confined and at that time the egg is more susceptible to lack of oxygen.

The rhythms of difference of potential may also have some relation to the processes of differentiation which Scott¹ found existed in the fertilized eggs of *Amphitrite*. These processes of differentiation may be started into activity at certain definite susceptible periods, by mechanical agitation. The periods are at 35 to 50 and at 80 to 100 minutes after the eggs are placed in sea-water, periods in which the polar bodies are extruded and the first segmentation furrow appears. Scott found that eggs subjected to mechanical agitation at these periods develop parthenogenetically in greater numbers than when agitated at any other time. In harmony with these phases of differentiation in the egg of *Amphitrite* are also the periods in the starfish egg which are highly susceptible to artificial fertilization. Delâge² found these periods to be between the breaking down of the germinal vesicle and the appearance of the first polar body.

It seems that phases of susceptibility to lack of oxygen or presence of potassium cyanide, and phases of differentiation or physical manifestations of change in the constitution of different eggs, all point to a relation to certain phases of the rhythms of differences of electrical potential found to exist in the eggs of *Fundulus*. In this egg the extrusion of the polar body takes place about 15 to 20 minutes after the egg is placed in sea-water. The first segmentation nucleus forms about 15 to 20 minutes after fertilization, and the segmentation furrow appears about 90 to 100 minutes after fertilization. At these

¹ SCOTT, J. W.: Biological bulletin, 1903, v, p. 35.

² DELÂGE: *Loc. cit.*

intervals of time it is seen from Table IV that the difference of electrical potential is greater at the blastodisc area. From these periods onward the difference of potential decreases, and the current is reversed. At the beginning of these phases of positive differences of potential, when the segmentation nucleus and the segmentation furrows are forming, it is probable that the viscosity or contractility of the protoplasm is increased. Moreover we learnt from Lyon's paper that during the phases which correspond to those of positive difference of electrical potential, as indicated in Table IV, the Echinoderm egg was least resistant to lack of oxygen and potassium-cyanide, and from Scott's observations that the Amphitrite egg was most susceptible then to mechanical agitation, since more eggs develop parthenogenetically at these phases than do when agitated at any other time. It is also during these phases that the chromatin is in its anabolic or synthetic stage, building up the complex nucleochromatin compound, and during this time it has its greatest affinity for oxygen. Delâge on the other hand found that the starfish egg was more susceptible to artificial fertilization between the time of dissolution of the germinal vesicle and the appearance of the polar bodies. During this period the viscosity of the egg's protoplasm is decreased and probably its irritability greatly increased.

A CONSIDERATION OF SOME THEORIES OF SEGMENTATION, AND
THEIR BEARING UPON THE DIFFERENCES OF ELECTRO-
MOTIVE FORCE IN EGGS.

It would be interesting to ascertain the factors which cause the difference of electrical potential, and to determine their relation to those factors assumed by the prevalent physical theories to effect segmentation of the egg. A consideration of the mechanical and chemical theories which attempt to explain maturation and segmentation of the egg, and of the physical phenomena upon which Lippmann's capillary electrometer is based, as well as important facts made known through the works of Bredig, Ostwald, Hardy, Van't Hoff and other physico-chemists, regarding colloidal solutions and ion actions, should direct to a better comprehension of the physical manifestations of the processes of cell division, as well as account for the existence of differences of electrical potential in developing eggs. I shall therefore briefly review some of the theories and investigations that bear on this problem.

One group of authors, in which may be placed Van Beneden,¹ Boveri,² Heidenhain,³ and Rabl⁴ considers the astral and spindle rays composed of contractile substances and extending from the centrosomes to the periphery of the cell, as the force that directs the processes of segmentation. In the other group are Bütschli,⁵ Rhumbler,⁶ Hertwig,⁷ and others. They support the dynamic theory, and regard the centrosomes as the dynamic or chemical centre that controls nuclear and cell division. The radial fibres or astrospheres, they believe, are dynamically induced formations of the centrosomes, or chemical modifications of the proximal protoplasmic elements. They are compared with lines of force, and regarded as visible expressions of the chemico-physical interaction of the central body and plasma.

Conkling⁸ inferred from his study of the segmentation of the egg of *Crepidula*, and V. Erlanger,⁹ from his observations on the segmenting eggs of the Nematodes, that a fluid state of the protoplasm was one of the important conditions for cell division. They noticed at each segmentation period a superficial streaming of the protoplasm from the poles toward the equator of the egg. In his physical explanation for cell division, Bütschli¹⁰ also assumes the existence of a fluid state of the protoplasm. He is of the opinion that the astral formation arose by tension action, associated with absorption by the centrosomes of exudations of nuclear fluid and the dissolved substances which diffused into the nuclear fluid from the plasma. The influence that the astral and spindle fibres exert at the equatorial plane, where the diffused substances from both astral phases meet, he believes must be greater than at any other part of the egg. Moreover he believes that segmentation furrows are produced by increased action

¹ VAN BENEDEN: Bulletin de l'Académie Belgique, 1887, xiv, p. 3.

² BOVERI: Verhandlungen der physikalisch-medicinischen Gesellschaft zu Würzburg, 1895, p. 29.

³ HEIDENHAIN: Archiv für Entwicklungsmechanik, 1895, i, p. 473.

⁴ RABL: Anatomischer Anzeiger, 1889, iv.

⁵ BÜTSCHLI: Archiv für Entwicklungsmechanik, 1900, x, pp. 54-57.

⁶ RHUMBLER: Archiv für Entwicklungsmechanik, 1896 and 1897, iii and iv, p. 659.

⁷ HERTWIG: Abhandlungen der Bayerischen Akademie, 1898, xix.

⁸ CONKLING: Biological Lectures, Wood's Hole, 1899.

⁹ V. ERLANGER: Biologisches Centralblatt, 1897, xvii, pp. 152-339.

¹⁰ BÜTSCHLI: Archiv für Entwicklungsmechanik, 1900, x, p. 52; Untersuchungen über mikroskopische Schaume und das Protoplasma, 1892.

of surface tension at the equator, due to strengthened activity at the place of the dissolved substances, and to the influence that the asters exert there. It is well known how Bütschli¹ attempted to demonstrate the action of the centrosomes by allowing warm gelatine oil-foam, in which spindle radiations had formed, to cool. The effect was a shrinkage of the air in the foam spaces, that in turn exerted a pull on the surrounding mass toward the centre of the spaces. A similar effect, he believed, was produced by the chemical and dynamical action that exists between the nucleus, centrosomes, and protoplasm, brought about by the absorption of water by the centrosomes from the surrounding mass. The withdrawal of water causes a shrinkage in the mass, and indirectly a pull or tension in the form of spindle fibres among the cytoplasm, at the time of mitosis. Loeb² agrees with Bütschli, Conkling, and Quincke in assuming that the phenomena of cell division are dependent upon protoplasmic streaming, which in turn requires a definite degree of viscosity. The viscosity is influenced by the presence of electrolytes. Moreover a change in surface tension also produces protoplasmic streaming; while, on the basis of Lippmann's observations, electrical changes must lead to changes in surface tension. Loeb imagines that diffusion currents exist at the surface of the egg which meet at the plane which separates the astral rays. These currents, he believes, lead to whirling movements that are symmetrically arranged to the equatorial plane. These whirling movements normally lead to segmentation producing increased surface tension at the equator, and decreased tension at the poles. When viscosity of the protoplasm is greatly increased through the concentration of the salt solutions, for instance, the diffusion currents and whirling movements are weak and will then not lead to segmentation.

Mrs. Andrews' observations on the living substances in the eggs of starfish and sea-urchins are in harmony with those published by Bütschli. Before maturation she saw contractile waves proceed to the dissipation of the nuclear membrane, preparatory to the formation of the egg nucleus. The cytoplasm then becomes more fluid, since the watery contents of the nuclear sac mingles with it. Later marked viscosity of the whole internal cytoplasm increases towards the periphery of the egg, while just before renewed division the mass

¹ BÜTSCHLI: *Loc. cit.*

² LOEB J.: This journal, 1902, vi, p-432; *Archiv für Entwicklungsmechanik*, i, p. 469.

of the egg would be more relaxed or fluid. During fertilization and cell division were to be seen never-ceasing series of redistribution and reorganization of the foam elements, and rhythms related to the cleavage of each cell. Besides the major rhythms of viscosity, every smallest area of the cytoplasm and nuclear substance has moreover rhythms of its own varying viscosity that bear a constant relation to the phenomena of cell division and karyokinesis, and are chiefly visible as local or astral modifications. In the sea-urchin's egg segregation of pigment granules at certain intervals of viscosity was suggestive, as correlated with structural preparations of certain areas for special physiological functions. Before each cleavage, pigment granules were carried along in the flux of the streaming substance toward the line where the split was to take place, and appeared to be carried outward and inward from this point.

In addition to all these observations, it must be added that attention was called by Fol,¹ years ago, to the resemblance of mitotic figures to those in a field of magnetic force, and later Zeigler and also Gallardo² pointed to this resemblance. Gallardo, by a very ingenious method, illustrated how the action of the centrosomes might produce the astral spindle radiations. He placed crystals of quinine sulphate in a vessel of turpentine into which were put two insulated wires connected with the poles of a condenser. The crystals oriented themselves like the lines of force in an electric field, and so were produced radiations at each pole, as well as a connecting spindle. On the basis of the various phenomena that he secured, he thought it possible to refer the force causing the mitotic figures to one emanating from the central bodies. He believed that the centrosomes cause radiations during segmentation, and that they act by some power similar to a magnetic or an electric force. Rhumbler,³ on the other hand, assumed in his earlier work that an electric force worked in unison with surface tension in cell division, but changed his view when he learned that Roux found that an electric current exercised no definite directive influence on the segmentation of the frog's egg. Rhumbler³ believed cell division possible through the influence of the contractile power of the radial fibres, and an increase

¹ FOL: *Jenaische Zeitschrift*, 1873, vii.

² GALLARDO: *Anales de la Sociedad científica Argentina*, Buenos-Aires, 1896 and 1897, xlii and xlii.

³ RHUMBLER, L.: *Archiv für Entwicklungsmechanik*, 1896, iii, p. 57, and 1897, iv, p. 713.

of the cell membrane, produced either by growth or by expansion, the latter as a result of the fluid given off from the nuclear sac at the period of dissolution of the nuclear membrane.

Roux¹ was of the opinion that segmentation was produced by electrical action, and argued that in this case an electrical current passed through a solution that contained frogs' eggs would influence the normal process of division. As the result of a strong constant current passed through the solution containing frogs' eggs, he observed a typical wandering of the pigment towards a polarized area of the egg. The outline of the polar area was on the anode side, and it is of great interest that in the blastula each cell shows its special polar field. When unsegmented eggs were subjected to weak currents, slight protrusions, extraovate-like formations appeared at each pole, but they did not possess the polar field. On the other hand, when the eggs were subjected to strong currents, the protrusions disappeared. In Tritons' eggs, the weak current produced a pigmented polar area, and the eggs became elongated in the direction of the current, while the current caused fertilized but unsegmented Teleosts' eggs to bulge out at each pole, so that a furrow was formed in the median area between the protrusions. The polar changes occurred at entrance and exit of the eggs, and only when the eggs were moist. Although Roux employed maximal currents for the frogs' eggs they did not seem to influence normal segmentation.

It is questionable whether Roux's observations gave Rhumbler sufficient or conclusive reasons for changing his first view, and reasons for doubting that an electric force acted in unison with changes in surface tension in effecting the segmentation of eggs. His results are not decisive in proving that electrical currents are not concerned in mitotic division of the nucleus, since such high local tension might be produced in the nucleus that currents which at their entrance would not act destructively on the cell body, might not be strong enough to influence the normal division of the egg. Even the strongest current may, as Roux points out, give negative results, since through its high tension it may have the tendency to include the surface of the conductor, and thus fail to penetrate the cell nucleus.

The protrusions or extraovate-like formations of unsegmented eggs acted upon by weak currents, indicate a localized lowering of the

¹ Roux, W.: *Gesammelte Abhandlungen über Entwicklungsmechanik*. 1895, i and ii, pp. 545-765.

surface tension, due to electrical influence, while the disappearance of these, when strong currents are applied, again show a change in surface tension, but this time an increase. These changes of surface tension and concomitant formation and disappearance of extraovate-like structures resemble the action of dilute and concentrated salt solutions on the development of Echinoderm eggs. In these, the segmentation is interfered with by the action of electrolytes that produce in dilute solutions extraovate blastomeres, while if these are now placed in concentrated solutions, the extraovate-like formations may disappear, but the normal segmentation of the egg will still be interfered with. I assume that the weak electrolytic solution changes the surface tension of the egg, the osmotic pressure and the state of its protoplasm, through its absorption of water, and the action of the electrically charged ions. The stimulating action of an electrical current is due to polarization, and since polarization in the frog's egg is well marked, we should have expected that the egg would be stimulated with a suitable strength of current or affected in such a manner that its normal development would be influenced. It is true that the eggs of some animals are resistant to sudden changes of osmotic pressure of the media in which they live. *Fundulus*, for instance, as Loeb¹ proved, develops in distilled water as well as in concentrated sea-water, being permeable to both, and it may be that this property interferes with the influence exerted by the electrical current, for Brown² showed that these eggs are polarized with difficulty, and that the current has no influence on their development, while *Arbacia* and *Asterias*' eggs are susceptible to electrical currents. I believe, therefore, that Rhumbler's earlier views deserve support, and that an electrical force acts in unison with surface tension in the segmentation of eggs.

A review of many of the hypotheses which account for the factors producing segmentation shows that most of them are in harmony in placing in the centrosomes and their radiations the central force for nuclear and cytoplasmic division, and in regarding them as the chief factors in the eggs' polarity. According to other theories, a change in state, or streaming condition of the protoplasm, and an alteration in surface tension over a localized area, combined with tension action of astral rays, are the principal agents in cell division.

Our next inquiry is whether during maturation and division there

¹ LOEB, J.: *Archiv für die gesammte Physiologie*, 1894, lv, p. 370.

² BROWN, O. H.: *This journal*, 1903, ix, p. 113.

are evidences of changes in the chemical composition and the reactions to stains of any of the constituents of the egg, definitely related to the varying physiological changes that accompany the segmentation of the egg, and which directly or indirectly add strength to the morphological and dynamical theories of segmentation.

As regards the function of the nucleus, it is the opinion, since Nussbaum's¹ experiments on infusoria, that the nucleus is the centre for anabolic and formative activity, and Korschelt's² experiments on the ova of *Dytiscus*, strengthened the fact that the nucleus was the most influential in synthetic and assimilative processes in the egg. Korschelt compared its influence on the surrounding substance to ferment action.

According to Kossel,³ Carnoy, and Lebrun,⁴ and others, the cell is composed of three organized proteid groups of substances. The composition of the nuclein group, for instance, varies in accordance with the different conditions of the nucleus, whether it is at rest or active during the processes of division. The nucleinic acid moreover is found in the nucleus in different states, first, as free acid, second, combined with a base, such as a protamine and albumose, and third in combination with albumin. According to Kossel, the albumose disappears in ripe sperm, and the protamine then combines with the nucleic acid to form nuclein.

From the above, and other related statements we conclude that the chemical constitution of the nucleus and the cytoplasm is modified during maturation and cell division, and that these changes are related to definite functions of the constituents of the egg. We may bring these facts into relation with the staining reaction of chromatin, by recording some of the observations made on eggs during the history of the germinal vesicle and the nucleus during segmentation.

Kossel⁵ and Lillienfeld⁶ proved that the nuclein series show an affinity for basic dyes in direct proportion to the amount of nucleic acid they contain. From the fact that chromatin in the cell nucleus assumes, during different phases of maturation and division, various

¹ NUSSBAUM: *Geschichte für Natur und Heil-Kunde*, Bonn, 1884.

² KORSCHOLT: *Zoölogisches Jahrbuch-Anatomische Abtheilung*, 1891, iv.

³ KOSSEL: *Verhandlungen der physiologischen Gesellschaft*, Berlin, 1892-1893, xvii-xviii, pp. 5-6.

⁴ CARNOY and LEBRUN: *La cellule*, 1899, xii, p. 197; 1885, i, p. 202.

⁵ KOSSEL: *Archiv für Physiologie*, 1891; 1893, p. 181.

⁶ LILLIENFELD: *Archiv für Physiologie*, 1893, p. 395.

shades of blue-green to green, with Ehrlich's methyl-green stain, and pure green, most intense during mitosis, Heidenhain¹ concludes that the proportion of nucleic acid and albumin varies with periodic changes in the nucleus, and that during mitosis the chromatin consists largely of nucleic acid. Since free nucleic acid possesses qualities which in combination with albumin disappear, the idea arose that the combination and dissociation of these indicate especially during mitosis important physiological processes, related to functions of the nucleus.

The changes undergone by the chromatin during the growth of the egg were also studied by Rückert.² His observations corroborate those mentioned above. He found that at an early stage the chromosomes are small and stain deeply; later they increase enormously in size, but lose their staining power. As the egg approaches its full size, the chromosomes decrease in size, but increase again in staining power. The variations in size of the chromosomes point, Rückert believes, to absorption of large quantities of matter, that produce combinations which do not take on the chromosome stain. When this matter leaves the chromosomes, as a result, possibly of katabolic changes, the chromosomes are left with greater staining power. He concludes further that increase of surface of chromatin facilitates exchange of material between chromatin and plasma. The high percentage of nucleic acid originally contained in the chromosomes gives to it its staining power, when, in combination with a large amount of albuminous substances, it forms lower members of the nuclein series. The resumption of staining power is caused by a decrease of albumin and restoration of nucleic acid preparatory to division. Greatest activity in cytoplasm coincides with decrease in nucleic acid and staining ability; while suspension of activity corresponds to richness of nucleic acid and greater power of staining. The chromatin passes through cycles in the life of the cell. During vegetative activity, there is an increase of albumin and a decrease in the reproductive phase of activity.

Mrs. Andrews also observed in the starfish's egg that at different stages in the changing optical and physical conditions the astral rays show differences of reactions to chemicals as characteristic of their substance in its varying states of viscosity or contraction. A few seconds may make a difference in reaction of these structures to hardening fluids.

¹ HEIDENHAIN: *Archiv für mikroskopische Anatomie*, 1894, xliii, p. 423.

² RÜCKERT: *Anatomischer Anzeiger*, 1892, iv, p. 107.

Other writers have advanced chemical theories. Strassburger for instance, assumes that the movements of the chromosomes may be chemotropic, and Carnoy believes that the asters are formed under the influences of specific ferments.

PHYSICAL CONDITIONS THAT INFLUENCE CLEAVAGE.

From the theories and facts which have been cited above, it would appear that the essential conditions for cleavage are an increase of surface area over a definite region of the egg; a flowing state of the cytoplasm; tension action of the astral radiations; directive force of the centrosomes; and metabolic control over the cell by the nuclear elements; moreover it would seem that these factors are related to chemical changes especially in the chromatin elements of the egg manifested at definite phases of mitosis by reactions to special stains. Chemical changes are, however, not necessarily the fundamental cause of the physical alteration of the surface extent of the egg, or of the phase of its cytoplasmic constituents during segmentation. It becomes necessary therefore to seek for the factors that will explain these conditions.

It is well known that the potential energy present at the surface of a liquid tending to occupy a smaller volume, produces a tension that is opposed by a force which tends to increase the size of the liquid surface. Of the forces that may alter surface tension, the first in importance is undoubtedly the energy manifested by the difference in osmotic pressure of electrolytes that may exist on the sides of the limiting surface. The effect of electrolytes on surface tension is easily demonstrated in living or artificially produced cells with thin semipermeable membranes. The enlargement of the cell by entrance of fluid that effects dissociation of contained electrolytes and increase of their solution tension, indicates a lowering of surface tension of the cell.

It is at present an established fact that the chief physiological effect of an electrolytic solution depends upon the number of its dissociated ions through the charges they bear. The works of Bugarszky and Tangl,¹ Hardy,² Bredig,³ Loeb,⁴ Mathews,⁵ and others

¹ BUGARSKY and TANGL: *Archiv für die gesammte Physiologie*, 1898, lxxi, p. 467.

² HARDY: *Proceedings of the Royal Society*, 1900, lxvi, p. 111.

³ BREDIG: *Anorganische Fermente*, 1901, p. 16.

⁴ LOEB: *This journal*, 1902, vi, p. 430.

⁵ MATHEWS: *This journal*, 1904, x, p. 431.

have proved that metals are most active in the ionic state, and that the ions act chiefly by means of their electrical charges. Mathews believes also that the effectiveness of the ions is determined by the affinity that the ion has for its charge.

The increase of surface over a definite region of the egg in cleavage, due to lowering of surface tension, can also be brought about by placing unfertilized eggs, *e.g.*, those of the starfish, for a time in hypoisotonic salt solutions, in which the eggs increase in size and form extraovates or dumb-bell shaped forms.

On the other hand, the cleavage of the cytoplasm, and increase in surface extent, does not appear if starfishes' eggs, or those of *Scyphomedusæ*, and other forms are placed in strong hyperisotonic solutions. It may be said that in the former case, in the more dilute solution, the dissociations of electrolytes into ions is more complete, producing an increase of electrical charges, sets free more thermoelectric energy, and also increases the stability of the colloids in the cytoplasm. These conditions tend to lower the surface tension, and produce the enlargement of the egg. In the latter case the surface tension is increased, not diminished, because the number of electric charges acting counter to the surface tension are decreased and the cytoplasm or hydrosol is rendered thereby more unstable. But a more satisfactory explanation may be drawn from researches conducted by Lippmann and Helmholtz,¹ who have demonstrated that surface tension at the plane of separation between a metal, *e.g.*, mercury, and an electrolyte is a function of the difference of potential of this plane, and that the force with which the surface tends to diminish itself is determined by the natural surface tension of the mercury diminished by the force with which the electric charges of the ions tend to increase the surface. If the metal is positive it carries positive charges, and this surface of charges lowers surface tension, in that the like ions repel each other and thus through their repelling force increase the surface area. The surface tension and difference of potential are altered by the addition or subtraction of electrical charges, and the potential between metal and electrolyte depends upon the solution tension of the metal and the osmotic pressure of the ions. The cause of difference of potential between a metal and a solution we have learned is on the one hand, the solution tension of the metal, tending to drive ions from the metal into the

¹ OSTWALD: *Lehrbuch der allgemeinen Chemie*, 1893, ii, p. 92 2.

solution, and the osmotic pressure of the solution, acting counter to this, tending to cause the kations already present to separate on the electrode in the metallic condition.

This explanation leads to the inference that the charges accumulating on each side of the semipermeable egg membrane during different phases of mitosis are the active agents in lowering the surface tension and responsible for the difference of electrical potential. Their relative proportion and signs depend upon the strength, reactions and nature of the electrolytes that are separated by the membrane.

Their presence at the periphery, the streaming state of the protoplasm, as well as the formation of the segmentation furrow next deserve our consideration. Bütschli believes that segmentation is due to a lowering of surface tension at the region nearest the asters. These structures, he maintains, repel each other, because they bear electrical charges of like sign. They are acid poles that repel ions of like sign centrifugally; this lowers surface tension over this special area, while at the same time the interaction of the ionic charges produces a streaming of the protoplasmic contents. It was observed by Mrs. Andrews, Conkling, Erlanger, and others that protoplasmic streaming may be distinctly seen during mitosis. The paths of movements are from the periphery toward the equatorial plane. In addition to these active manifestations, Mrs. Andrews noticed rhythms of incessant changes of viscosity in the protoplasm, the dissolution of the nuclear membrane and the liquid contents of the nuclear sac diffusing into and increasing the dilution of the cytoplasmic fluid. The question is, what are the physical explanations for these phenomena, and what is their significance?

According to Hardy¹ and Bredig² protoplasm is a colloidal solution consisting of solid particles in a fluid, the particles floating in a dilute solution of the colloid and water and possessing a double electrical layer at the limit between the suspended particles and the fluid. The viscous solution surrounding the particles resists their movements through the fluid; moreover, they are kept apart by electrical charges of the same sign which they carry, the charge being of opposite sign to that of the surrounding medium. The colloidal particles may carry either positive or negative charges depending

¹ HARDY: *Loc. cit.*

² BREDIG: *Loc. cit.*

upon the reaction of the surrounding medium, that is, the proteid particles and the fluid form an electrically homogeneous mass when the fluid is neutral. On the other hand, the proteid particles have electrical characters conferred upon them by the nature of the reaction of the fluid. The proteid particles seem to act as basic or acid particles, according to the circumstances in which they find themselves. Under the influence of an electrical current, they go to the kathode in an acid, or to the anode in an alkaline medium. If their charges are taken from them either by oppositely charged ions or an electric current, their physical nature is altered; they are converted from hydrosols into hydrogels. The stability of the system may also be destroyed by induction, the active agents being free ions carrying static charges. In this case the action is on the electric layers immediately around them, and the active ions are those whose electric sign is the opposite to that of the charge on the surface of the particles. By robbing them of their charges they reduce their surface and produce protoplasmic movements. Consequently salts and alkalies, as well as acids, if present in a solution with suspended colloidal particles may act on these, the result being decomposition of complex molecules. Anabolic as well as katabolic changes may thus occur as a result of electrical charges which, even in small quantities may produce great effects, for as Bodlander has shown, one part of hydrochloric acid in 1500000 parts water was effective in producing marked precipitating changes in suspensions of different substances, resembling catalytic action.

The change in state and streaming of protoplasm is accordingly regarded as due to the interaction of the electrical elements. That is, the ions either rob compounds of their charges, or are deprived of theirs by the compounds, so that new affinities are developed and new relations produced among the fluid and solid constituents of the egg's contents. Besides the difference in surface tension that obtains among the particles of the egg protoplasm, there must exist a difference of electrical potential and surface tension, not only between the surfaces of nuclearplasm and cell protoplasm, separated by the nuclear membrane, but also between the surface of the egg and its surrounding medium. The semipermeable membrane separating electrolytic solutions may be compared to the two parts of a Franklin condenser separating on the one side positive charged ions from the negative on the other, and thus surface tension comes into play. The nature of the membrane and its resistance to influences

may be affected by its surrounding electrolytes, and therefore changes in solution tension of its own component particles may arise, and secondarily, have an effect upon the surface tension as well as on the difference of electrical potential of the egg.

We are led to infer that the chemical and physical energies manifested among the mass of heterogeneous ions, atoms, and compounds in the egg's protoplasm and its surrounding fluids produce the vital phenomena of development. At the time of maturity a certain equilibrium of forces has been established. With the entrance of the spermatozoon, the existing state of equilibrium is upset. The forces between the electrolytes and colloids receive a new impetus that starts activities which lead to segmentation.

The combined male and female nucleus is the pole that exerts the directive force, representing, as it were, in its function an automatic centre which is kept active by the interaction of its own ions and those of its medium. It may therefore effect oxidation and reduction changes or synthetic and analytical processes.

We know from chemical analysis and microchemical tests made by Kossel, Rückert, Lebrun, and others that the chromatin molecule decomposes during its active phase, setting free nucleic acid and albumins, and during the resting stage, the nucleic acid, albuminous and protamine groups combine again with each other. During the katabolic acid stage the chromatin particles therefore carry charges whose signs are opposite to those carried by them during the neutral or alkaline anabolic phase. The chromatin particles would move therefore under the influence of an electric current to different poles, wandering with its negative charge in an alkaline fluid during the acid phase to the anode and to the kathode during the alkaline phase. Inductive action would also exert its effect, producing a change in the state of the charged ions by acting on the electrically charged double layer, causing a repulsion or attraction between them and the acid chromatin particles.

I would agree with Bütschli, Lillie, and others, that during the acid phase the centrosomes resemble highly concentrated electrostatically charged poles that are not in a field of force only, but also in an electrolytic field repelling the ions of like sign toward the equator, while attracting those of unlike sign. At the periphery of the egg there would exist therefore an electrical double layer of ions bearing negative charges inside of the membrane and positive charges outside. In the meantime the interaction of the electric charges of the

ions forming the acid pole with those attracted by them carrying charges of opposite sign, accompanied by the absorption of fluids, produces a change in the physiological function of the polar chromatin elements. At the same time the ions that were repelled toward the equatorial plane meet in an electrolytic medium of definite osmotic pressure and it is possible that new combinations are here also formed. As a result of the change progressing at the poles and equatorial plane, chromatin elements of each half of the plane not only repel each other, but are also attracted toward the astral poles; the latter in consequence of their physical and chemical changes, have reversed their electric surface charges. At the equatorial plane, there is produced therefore a field of stress that greatly increases the surface tension over a definite area of the egg, and thus instigates the formation of the segmentation furrow. The complex forces that have been developed, produce at first dissolution of the nuclear membrane and a less viscous and more irritable state of the cytoplasm; moreover, some of the colloidal elements of special composition arrange themselves in the field of force as precipitated fibril strands. The formation of the astral radiations are due, according to Harvey, to a pre-existing stress that fashions the foam-network of the cytoplasm, so that they are a coarse diagram of a dynamic phase of the cell's history. He bases his statement on the results that he and also Bütschli obtained from albumin and soap films that were subjected to a stress. By fixing the film in alcohol the threads of coagulated albumin could be distinctly seen as fibres radiating from the centre of application of stress.

We should expect that the changes in surface tension produced indirectly by the interaction of the centrosomes and other elements of the egg would be accompanied by changes in differences of electrical potential between the surface of the egg and its external medium, and in mesoblastic eggs between the vegetative and animal poles. The difference of electrical potential, if present in a measurable degree, should be detected therefore with a suitable galvanometer, as the different stages of mitosis and the changes in intensities of the acid and alkaline phases make their appearances.

The observations on the differences of electrical potential secured from the turtle's egg during maturation, and from *Fundulus* eggs after fertilization, prove that the phases of mitosis are accompanied by changes both in the character and the number of the surface charges born by the egg. During the maturation period the surface

of the blastodisc in the turtle's egg is at a higher potential than any other part of the egg's surface. Moreover, one pole of the blastodisc near the nucleus has a greater difference of electrical potential than has the opposite pole. The sign of the surface charge is the same as that existing on the surface of the blastodisc of the *Fundulus* egg at about twenty minutes after fertilization, and again when the segmentation furrow is forming. When the egg is placed in the electrometer circuit at these periods, a current is indicated as passing from the animal pole of the egg toward the vegetative one. During the time intervening between these periods the surface bears charges of opposite signs and the direction of the current in the electrometer and the egg is reversed. According to the argument advanced above, the chromatin during this interval of time is undergoing decomposition and is progressing in its katabolic acid stage. In this state it attracts oppositely charged ions and repels those carrying charges of like or negative sign to the periphery, thereby lowering its own surface tension, while the preponderance of these charged ions, by inductive action, attract to the outer surface of the egg electrolytes that carry positive charges. If these statements are in accordance with the facts then we should expect that if the egg were placed in contact with the non-polarizable electrodes of the electrometer during the acid phase of the chromatin, the mercury in the electrometer, which, in contact with the sulphuric acid, is positively charged, would receive additional positive charges from the surface of the egg. These charges would increase its original charge and consequently decrease the surface tension of the mercury, causing it to drop from the capillary tube of the electrometer, thus indicating a current that travels in the circuit through the egg from the vegetative toward the animal pole. The greater the number of charges on the surface of the egg, the lower is its surface tension, and the greater is the extent of the movement of the mercury in the capillary. On the other hand, when the surface charge of the egg is negative then the mercury receives negative charges which decrease its surface charges and increase its surface tension, so that now the mercury rises in the capillary, indicating a current in the egg, if it is placed in the circuit from the animal toward the vegetative pole of the egg. From Table I and also Table IV, it is seen that this is just what occurred. If this is true, then we may say that the change in differences of electrical potential is a reliable index of the changes in surface tension, streaming state of the protoplasm, and other

special chemical and physical alterations going on in the egg during its mitosis. The difference of electrical potential is difficult, if at all possible, of detection in eggs that possess a thickened egg membrane, or one complicated by accessory structures or secretions that could interfere with the ready interchange of ions between the interior and exterior of the egg. The interchange of electrolytes is also impeded by the more viscous, less irritable state of the egg's protoplasm, due to absorption of the cytoplasmic fluid, or to diffusion produced by a high external osmotic pressure. The conditions that control the segmentation of the egg may be determined by mass action or by the interaction of enzymes, both of which are dependent upon the electrical or physical character of the complex molecules or compounds that are prepotent at any given time in the egg. Whether two substances combine or remain inactive at a definite temperature, it is well known, depends upon the concentration of the substance. It is possible that at a certain concentration they are completely dissociated, while at any other only partly so. According to the law of Guldberg and Waage, the tendency and rapidity for chemical reaction of a substance is determined by its mass action, that is, by the concentration of the reacting substance, or the substance acted upon, or the reaction product. The greater the amount of reaction substance that has been formed, the greater is the tendency to reverse the action. It has also been demonstrated that the reaction is reversible in all cases in which the mass action is discernible, that is, in which the reaction is incomplete, in contradistinction with the usual complete reaction, as, for instance, in saponification of an ester by an alkali, and in certain catalytic actions, *e. g.*, the catalysis of ethyl acetate, water, and acid which is opposed by the catalysis of a mixture of equimolecular quantities of the reaction products. The reversible action of enzymes also depends upon the presence of the reaction products. This fact has been demonstrated, since Croft Hill's experiments, by others, and lately by Loevenhart and Kastle.¹ The behavior of the reversed chemical and catalytic actions may perhaps be inferred from the behavior of the secondary or reversed movements of suspended proteid particles that were subjected to an electrical current. They were, as Hardy² observed, negative to the water, and became positive to it after a longer or shorter sojourn in the neighborhood of the anode. This change may be referred to the

¹ LOEVENHART: This journal, 1902, vi, p. 331.

² HARDY: Journal of physiology, 1899, xxiv, p. 288.

electrolytic action of the current upon the electrolytes present. Owing to this, an electronegative particle which moved to the anode would there reach an acid region in which its character would change from electronegative to electropositive. This view is supported by the observation that the reverse movement is more pronounced, the higher the concentration of the electrolytes, and therefore the greater the concentration of acid or alkali at the poles. We assume that the molecules of a substance in solution exist under a definite pressure, and that every substance will pass into solution until the osmotic partial pressure of the molecules in the solution is equal to the solution tension of the substance. If a metal is put into one of its salts, the solution tension may be greater than the osmotic pressure, and metallic ions with their positive charges will pass into solution, which then becomes positive, the metal negative. At the plane of contact is the electric double layer. The positive charged ion in the solution and the negative charged metal attract one another and develop a difference of potential. The solution tension tends to force more ions in solution, while the electrostatic attraction of the double layer is opposed to this. When these forces are equal, the equilibrium is established.

If the solution tension is less than the osmotic pressure of the metallic ions in solution, then the ions separate from the solution. Palmaer¹ has demonstrated this by placing mercurous nitrate in a vessel whose bottom is covered with metallic mercury. On account of the low solution tension of the mercury, some mercury ions from the solution will give up their positive charges to the mercury, which in turn will attract electrostatically negative NO_3 ions to form the double layer. This will continue until a certain difference of potential has been reached, when equilibrium will be established. If a drop of mercury now falls into the solution, some mercury ions will separate upon it, charge it positively, and it will attract some negative NO_3 ions, and drag them down to the mercury. But when the drop unites with the mercury at the bottom, it will contain an excess of positive electricity, and the same number of mercury ions will pass into solution as negative NO_3 ions were carried down from the top.

The solution tension and osmotic pressure represent two forces that are ready to act at the moment the one or the other obtains the ascendency. The solution tension and the osmotic pressure of metals or complex compounds in a solution may be influenced by an elec-

¹ PALMAER: *Zeitschrift für physiologische Chemie*, 1899, xxviii, p. 287.

trical current, temperature, nature of the electrolytes present, and by the absorption or diffusion of liquids.

It is probable that during the development of the egg, processes of mass action, enzyme or catalytic reactions, and differences of electromotive force, all play an active part. They are controlled by the solution tension, and the osmotic pressure of the colloidal and electrolytic constituents of the egg. Of these elements, those of the chromatin group are the most influential. A certain state of equilibrium is reached when the ovum is ripe. When the sperm enters, it introduces definite ions that bring about an unstable condition and start new reactions that tend toward a new state of equilibrium. The combinations which the chromatin elements form depend upon the concentration of the electrolytes, the presence of reaction substances or reaction products, and enzymes in the cytoplasm. They carry charges of definite signs and number, and during certain phases in the development of the egg pass through reversed action periods characterized as anabolic or neutral, and katabolic or acid phases. The chromatin particles are the most prepotent, and exert, through the action of their charges, the directive force in the phenomena of segmentation.

The views expressed in this paper regarding the physical factors that influence mitosis are more or less held by others, especially Bütschli, Gallardo, Loeb, and R. S. Lillie.¹ When this work was undertaken, I had the idea that segmentation was the result of special physical reactions herein mentioned. I communicated my views during the summer of 1902, while at work at Wood's Hole, to Dr. F. R. Lillie and Dr. E. B. Wilson. Though these views are no longer entirely new, I publish them in their imperfect form with the results of my work, hoping that additional facts will be forthcoming that will establish the theory of segmentation more satisfactorily.

CONCLUSIONS.

In considering what has been said above in regard to surface tension and its effect upon mitosis, I conclude that the segmentation and the normal course in the development of the *Scyphomedusa* egg, placed in strong salt solutions, did not proceed because of the changes instituted in the egg by the more concentrated medium. The latter must have produced a difference in osmotic pressure between the

¹ LILLIE, R. S.: Biological bulletin, 1903, iv, p. 175.

salts in the egg and those in the water surrounding it, a withdrawal of water from the egg's contents, a lowered dissociation of ions, and an increase in the viscosity of the cytoplasm. Possibly in the more concentrated state of the electrolytic solution in the egg, certain colloid elements are precipitated. This is assumed from the investigations conducted especially by Schulze,¹ Linder and Picton,² Hardy,³ Bredig, and Pauli⁴ who have studied the precipitation of colloids by electrolytes. Therefore, in consequence of the concentrated medium, there occurred a decrease in the surface tension of colloids in the egg, and an increase in the surface tension of the egg itself. These phenomena would depend upon electrical action of ions that greatly alter the surface tension between colloidal particles and their fluid medium, as well as the differences of electrical potential that exist between the colloids and their surrounding electrolytic solution in the egg on the one hand, and on the other between that of the surface of the egg and its external medium. This statement is in accordance with Bredig's views which are based on the investigations of Quincke, Coehn, and others. They believe that suspensions and colloidal hydrosols possess electrical differences of potential toward the surrounding medium, and from the works of Lippmann, Ostwald, Rothmund, and others that the surface tension of two contiguous mediums is a function of their potential difference and that this, as well as the surface tension, may be greatly altered by the addition of certain ions.

In the maturing turtle's egg, a difference of electrical potential which increases as development progresses exists between the animal and vegetative poles, and also between the poles of the longer axis in the blastodisc. This fixed polarity is related to a definite axis of the egg as well as to one in the blastodisc.

Differences in electrical potential exist between the animal and vegetative pole in the fertilized egg of *Fundulus*. During the phases of segmentation these potential differences appear in periods of rhythmical sequence that are characterized by currents flowing for a definite time in one direction, gradually increasing to a certain limit and then decreasing, followed by a reversal of the current, which also increases gradually and then decreases. About twenty minutes after

¹ SCHULZE: *Journal für praktische Chemie*, 1883, xxvii, p. 320.

² LINDER and PICTON: *Journal of the Chemical Society*, 1895, lxvii, p. 63.

³ HARDY, W. B.: *Proceedings of the Royal Society*, 1900, lxvi, p. 115; *Journal of physiology*, 1899, xxiv, pp. 158-297.

⁴ PAULI: *Archiv für die gesammte Physiologie*, 1899, lxxviii, p. 315.

fertilization, and at the time when the segmentation furrow appears the electrical potential is greater at the animal pole, and at a certain time between these intervals it is least over this region. The periodic variations of electromotive force bear a definite relation to the resting alkaline anabolic, and active acid katabolic phases of the chromatin mass; in other words, to the different phases that are discernible during mitosis. They may also bear a relation to the rhythms of viscosity, resistance to pressure, and fixatives, as observed by Mrs. Andrews in the living protoplasm of the starfish egg, to the periods of resistance to oxygen and potassium cyanide, the production of carbon dioxide, ascertained by Lyon to obtain in the eggs of *Arbacia*, and to the intervals susceptible to mechanical agitation found by Scott to exist in the *Amphitrite* egg, moreover to those phases susceptible to artificial fertilization ascertained by Delâge to occur during segmentation in *Arbacia* eggs. The difference of electrical potential registered in the electrometer, when the egg is in the electrometer circuit, indicates that during definite intervals, as mitosis progresses, the surface of the egg carries charges varying in number and sign. They exist on the external surface of the egg as a result of electrostatic force, and bound one side of the Helmholtz electric double layer which exists between two fluid media, external and internal to the membrane. The electrically charged ions internal and external to the bounding surface are of opposite sign. The forces that produce these surface charges emanate from the centrosomes, and as a result of the interaction of electrical or physical elements of the chromatin, and also between it and the constituent particles of the surrounding colloidal and electrolytic solutions. Metabolic activities accompanied by the decomposition of complex molecules, or the construction of these, produce during the katabolic phase an acid chromatin mass that liberates and attracts charged ions. As a consequence of the interaction of ionic forces in the egg and its environment, chemical and physical changes result in the constitution of the egg's protoplasm.

It is possible that changes in osmotic pressure, solution tension, mass, and enzyme action, as well as surface tension, obtain during the egg's history. These produce alterations in the viscosity of the cytoplasm, absorption of liquid by the chromatin or cytoplasmic particles, and a shrinkage in the one or the other during definite phases of cleavage, the index for the variations of changes being the difference of electrical potential.

When a certain state of equilibrium is attained by the chromatin mass, a reaction started between it and its products sets in. It takes the form on the one hand of synthetic, on the other, of analytical processes; this is accomplished either by the building up of more complex chromatin molecules of a definite chemical reaction, or by the dissolution of the complex to a more simple compound of opposite chemical reaction. These alterations and interactions of ions and their associated energy elements are accompanied by physical and chemical changes of the egg's substances. These changes give expression to their dynamical or tension action in the form of astral and spindle radiations, dissolution and degeneration of the nuclear membrane, and chromatin molecules, and to the formation of cleavage furrows.

THE AUTOLYSIS OF ANIMAL ORGANS.—II. HYDROLYSIS OF FRESH AND SELF-DIGESTED GLANDS.

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ON hydrolysis of proteid material by mineral acids various crystalline products result. The same or nearly the same products appear on digestion of proteids by proteolytic enzymes of the gastric and pancreatic glands, and nearly the same substances on prolonged autolysis of animal glands and tissues. On the ground of these findings a general conclusion was drawn that most tissues contain an enzyme acting in the same manner as trypsin. Upon closer observation, however, it was noted that among the end-products of autolysis of animal tissues, substances occur which could not be obtained by cleavage of the tissue constituents by means of mineral acids, while some substances usually appearing on hydrolysis by means of mineral acids are found in small quantities, or not at all, among the products of autolysis. Thus Emerson¹ found, on self-digestion of the pancreas, oxyphenylethylamin and cadaverin. Kutscher could find only one hexon base—lysin on the autolysis of the thymus; Dakin isolated only one purin base after prolonged autolysis of the kidney. Lawrow² found, on self-digestion of the gastric wall, putrescin and cadaverin instead of lysin and arginin.

The end-products of the autolysis of the liver contain only one hexon base,—lysin; and those of the testes, only one basic substance,—hypoxanthine.

Several explanations of this difference are possible: the enzymes of the various organs may be unable to cause a complete cleavage of their tissues, in which case the products of self-digestion, if heated with strong mineral acids, should form the substances which failed to appear on autolysis. The other explanation may be that the same

¹ EMERSON: Hofmeister's Beiträge, 1901, vi, p. 501.

² LAWROW: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 312.

substances are formed during autolysis as on cleavage with mineral acids, but the substances undergo further decomposition, perhaps through the action of other than proteolytic enzymes. It is possible also that all the end-products suffer a quantitative change, either being decomposed or synthesized into more complex substances. That surviving tissues are capable of this double function was demonstrated by Wiener in regard to uric acid. The difference may also be explained by the supposition that tissues contain, besides the proteids already isolated and analyzed, substances which as yet have not been studied.

Thus, it seemed important in order to investigate the nature of the chemical reactions occurring in surviving tissues to compare the end-products of autolysis not with cleavage products of pure chemical substance, as individual proteids or nucleins, but with the cleavage products of the entire organ or tissue. This was the plan of the present work. The investigation, as yet, is not completed, and the results obtained thus far are communicated at present partly for the reason that while the work already was in progress, the very important investigations of Kossel¹ on Argynase, and of Walter Jones² on the Self-digestion of Nucleo-proteids have appeared. Their observations coincide with those made in course of this work and explain some of the findings.

The great part of tissues, and of protoplasm in general, consists of simple and of combined proteids. Proteid at large is a very complex molecule containing radicles with very different chemical properties. From the physiological point of view they all may be classified in the following groups: (1) nitrogenous acids (monoaminoacids); (2) urea group (arginin, lysatinin); (3) uric acid group (purin and pyrimidin bases); (4) chromogenic group (pyrol-derivatives); (5) sulphur group (cystin); (6) basic group (ammonia, lysin, ornithin); and (7) carbohydrate group.

The present communication represents only the results of the analysis of the basic constituents of the pancreas, spleen, and liver. The results of similar investigations of muscle will follow soon. The analysis of the amino-acids is in progress also.

¹ KOSSEL: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 321; 1904, xlii, p. 181.

² JONES: *Ibid.*, 1904, xli, p. 101; 1904, xlii, p. 38.

PANCREAS.

Purin bases.—The literature on the subject of transformation of the nuclein bases (purin and pyrimidin bases) in course of autolysis is reviewed by Jones in his recent publication, and therefore it will suffice to communicate here the results of this investigation. Four bases of the purin group had been identified in this gland; namely, adenin, guanin, xanthin, and hypoxanthin.

In the analysis of the fresh glands guanin was found in predominating quantity, adenin followed, and xanthin and hypoxanthin were not detected. If present, their quantity surely was very insignificant.

In the digested gland, on the contrary, little guanin was found. Adenin was totally absent, while xanthin and hypoxanthin were present in very appreciable quantity. The total sum of purin bases was markedly diminished in the digested glands. This observation is in harmony with that of other observers. Thus, Kutscher analyzing the purin bases of self-digested pancreas observed that the usual four bases were present among the products of digestion, though seemingly in smaller quantity than from the fresh gland. Burian and Hall noted the transformation of imid-purins into oxypurins in the pancreas. The difference in the results of the present experiment and of that of Kutscher may be explained by the fact that extracts were employed in the older work, while the entire gland was used in the present investigation.

Experimental part.—Five pounds of fresh glands were prepared in the usual manner, taken up in a 5 per cent solution of sulphuric acid and heated 12 hours, with return condenser, in a boiling water-bath. The product was cooled, filtered, treated with Hopkins' mercuric sulphate solution, and allowed to stand twenty-four hours. The precipitate was filtered on a suction funnel and washed with a 5 per cent solution of sulphuric acid. The precipitate was suspended in water and decomposed by sulphuretted hydrogen. The filtrate from mercuric sulphide was concentrated to remove the hydrogen sulphide, neutralized with ammonia and concentrated to a small volume. A precipitate formed consisting largely of guanin. It redissolved in boiling diluted sulphuric acid. The solution was decolorized by means of charcoal, filtered, and allowed to stand over night. Guanin sulphate crystallized. The sulphate was redissolved in boiling diluted sulphuric acid, transformed into the free base by means of

ammonia, and filtered while hot. The free base was again transformed into the sulphate, and that again was converted into the free base and analyzed.

0.116 gm. of the substance was used for a Kjeldahl's nitrogen estimation. 36.50 c.c. of $\frac{N}{10}$ H_2SO_4 were required.

For $C_5H_5N_5O$:

Calculated :	Found :
N = 46.36 per cent.	45.95 per cent.

The filtrate from the crude guanin and all the mother liquids of guanin sulphate were combined, and the purin bases contained in them were removed by means of an ammoniacal solution of silver chloride. This precipitate was filtered on suction and funnel, and decomposed by means of hydrochloric acid. The filtrate from the silver chloride was treated with a saturated solution of sodium picrate. The picrate was filtered while the mother liquid still remained warm. It was then recrystallized out of water, filtered on suction, washed with alcohol and ether, dried in toluol bath, and analyzed.

0.1170 gm. of the substance gave on combustion 0.1550 gm. CO_2 and 0.0247 gm. H_2O .

For $C_5H_5N_5C_6H_2(NO_2)_3OH$:

Calculated :	Found :
C = 36.26 per cent.	36.12 per cent.
H = 2.19 "	2.34 "

From the filtrate of adenin picrate, the picric acid was removed by means of sulphuric acid and ether. The purin bases remaining in solution were removed by means of an ammoniacal solution of silver chloride. The precipitate obtained in this manner was very small, and it was considered best to continue the further separation of the bases by Kossel's older method. The salt insoluble in cold nitric acid of sp. gr. 1.1 did not have the composition of hypoxanthin silver nitrate.

0.1472 gm. gave 0.0495 of Ag.

For $C_5H_4N_4O$, $AgNO_3$: For $C_5H_5N_5O$, $AgNO_3$:

Calculated :	Found :
Ag = 35.29 per cent.	33.65 per cent.
	33.70 per cent.

The mother liquid of the insoluble silver nitrate was rendered alkaline by means of ammonia, and only a very small precipitate formed. This precipitate might have been xanthin.

In a second experiment 10 lbs. of the gland were treated in the same manner. Guanin and adenin were obtained. The filtrate from adenium picrate was acidulated with hydrochloric acid, and allowed to stand over night. A small crystalline deposit was found. This was dissolved again in hot water, cooled, and acidulated with nitric acid, and shaken in separatory funnel with ether.² The nitric acid solution, free from picric acid, was allowed to stand over night. Again a crystalline deposit giving a positive xanthin test formed. Therefore the substance might have been xanthin.

The remaining mother liquid of adenin picrate was acidulated with hydrochloric acid, and extracted with ether. When all the picric acid was removed, the solution was concentrated under diminished pressure, rendered alkaline by means of ammonia, and treated with an ammoniacal solution of silver chloride. The precipitate thus formed was filtered on suction funnel. The silver salts were decomposed by means of hydrochloric acid. The excess of acid was removed by repeated evaporation of the solution under diminished pressure. The residue was twice taken up with alcohol and evaporated to dryness. The residue finally was treated with warm distilled water. The insoluble part did not give the nitrate characteristic of xanthin. The soluble part was treated with picric acid. A slight precipitate resembling adenin picrate formed. On standing, no other precipitates formed. Thus, if hypoxanthin was present, its quantity was very small.

Digested glands.—5 lbs. of the glands were taken up in 0.25 per cent sodium carbonate solution and allowed to digest ten weeks, toluol and chloroform being added to prevent bacterial growth. The products of digestion were concentrated to the original weight and decomposed with a 5 per cent solution of sulphuric acid in the same manner as with the fresh glands. The purin bases were obtained also by the same process as was employed with the fresh glands. The precipitate forming on concentration with ammonia gave a very small yield of guanin sulphate. This was transformed into the free base. It had the following composition.

0.0765 gm. of the substance gave 31 c.c. of nitrogen (over 50 per cent KOH) at $t^{\circ} = 24.5^{\circ}$ C. and $p = 760$ m.

For $C_6H_5N_6O$:

Calculated:
N = 46.36 per cent.

Found:
46.71 per cent.

The mother liquid of guanin sulphate was rendered alkaline with ammonia, acidulated with acetic acid, and filtered while hot. On cooling, a precipitate containing 39 per cent of nitrogen formed. It was dissolved in normal sodium hydrate solution. On addition of an excess of nitric acid (2 parts of acid to 3 parts of water), a precipitate formed. The precipitate was filtered on suction funnel, dissolved in ammonia water, precipitated by acetic acid, washed with alcohol and ether, and dried in xylol bath. It had the following composition:

0.1310 gm. of the substance gave on combustion 42.5 c.c. of nitrogen (over 50 per cent KOH) at $t^{\circ} = 26^{\circ}$ C. and $p = 760$ m.

For $C_5H_4N_4O_2$:

Calculated:	Found:
N = 36.84 per cent.	36.98 per cent.

The mother liquid from the crude guanin was treated with an ammoniacal solution of silver chloride. The silver salts thus obtained were decomposed with hydrochloric acid, and the filtrate from silver chloride was treated with a saturated solution of sodium picrate. No precipitate formed immediately. On standing forty-eight hours, a heavy crystalline precipitate was deposited. It was once recrystallized out of hot water, washed with alcohol and ether, dried in toluol bath, and analyzed.

0.1280 gm. of the substance gave on combustion 30.0 c.c. of nitrogen at $t^{\circ} = 28^{\circ}$ C. and $p = 762$.

For $C_5H_4N_4OC_6H_2(NO_2)OH$:

Calculated:	Found:
N = 26.85 per cent.	26.71 per cent.

The mother liquid of this precipitate was acidulated with hydrochloric acid, the picric acid extracted with ether, and the solution rendered alkaline by means of ammonia. The purin bases still present were precipitated by means of an ammoniacal solution of silver chloride. A very small gelatinous precipitate formed. It was treated by the method of Kossel.

The part insoluble in nitric acid of sp. gr. 1.1 had the following composition:

0.3030 gm. of the substance gave 0.1060 gm. of Ag.

For $C_5H_4N_4O$, $AgNO_3$:

Calculated:	Found:
Ag = 35.29 per cent.	34.98 per cent.

The filtrate from hypoxanthin silver nitrate gave on neutralization with ammonia only a very small precipitate.

Before reviewing the comparative yields of the bases, it may be well to bear in mind that it is impossible to obtain the individual bases in a sufficient degree of purity without loss of the substance, and that the quantities obtained in this work are regarded only as approximating those actually present in the tissues.

The comparative yields calculated for 5 lbs. of the gland were as follows:

	Fresh gland:	Digested gland:
Adenin:	4.0 gms.
Guanin:	2.6 "	0.200 gm.
Hypoxanthin:	trace	0.56 "
Xanthin:	trace	1.2 "

Pyrimidin bases.—Kutscher found hystidin on self-digestion of the gland, and the author isolated thymin, uracil, and cytosin from the cleavage products of the pancreas-nucleic acid. On self-digestion of the gland uracil also was obtained, the presence of cytosin was made probable, but thymin could not be detected. Further, it was established that pancreatic extract does not possess the power of transforming thymin into uracil. Thus, the difference in the pyrimidin bases obtained on autolysis of the gland, and on cleavage of the pancreas-nucleic acid, remained without explanation.

It was also very surprising that on acid hydrolysis of the gland, the pyrimidin consisted chiefly of uracil and of cytosin, thymin not being detected at all. This occurrence could be explained best by the assumption that there is more than one nucleic acid present in the pancreas, one yielding on cleavage the three known pyrimidin bases, the other containing no thymin, but uracil either as the only base of that group, or in a preponderating quantity. Of course there is also a possibility that in the presence of the large quantity of proteid material, and of other tissue constituents, the nucleic acids are affected by boiling mineral acid in a different manner than are free nucleic acids. This supposition, however, has to be abandoned for the reason that on acid hydrolysis of the spleen only thymin and cytosin and no uracil at all could be detected.

Of course the possibility is not excluded that both uracil and cytosin are secondary products, and that the gland contains more of the mother substance than does the free nucleic acid. Doctor Stookey and myself are engaged in the investigation of the nucleic

acid of the pancreas, with a view to ascertain the presence in the gland of more than one nucleic acid.

On hydrolysis of the fermented gland only uracil could be identified; also cytosin was absent. It thus seems probable that cytosin is transformed into uracil in course of self-digestion of the pancreas.

Experimental part.—The glands were heated with strong hydrochloric acid over direct flame with return condenser for twelve hours. The melanin formed during this cleavage was removed by filtration, and the filtrate condensed under diminished pressure. The residue was re-dissolved and reconcentrated repeatedly. The remaining hydrochloric acid was removed by means of lead carbonate, and the excess of lead by sulphuretted hydrogen. The filtrate from lead sulphide was concentrated under diminished pressure until crystals of tyrosin began to appear. The tyrosin was removed by filtration, and from the remaining liquid the pyrimidin bases were removed by silver and barytic water following the methods of Kossel-Jones and Kossel-Kutscher.

The precipitate containing the silver salts of the bases was taken up in sulphuric acid, the silver and sulphuric acid removed in the usual manner, and the remaining liquid concentrated under diminished pressure to a very small volume. On standing, a crystalline sediment formed. It consisted chiefly of uracil and of some cytosin. The mother liquid was treated with a saturated picric acid solution, and allowed to stand over night. A deposit of cytosin picrate was thus formed. The uracil precipitate was recrystallized out of a hot 2 per cent solution of sulphuric acid.

The substance thus formed had the following composition:

0.1600 gm. of the substance were used for a Kjeldahl nitrogen estimation. It required 28.5 c.c. $\frac{N}{10}$ H_2SO_4 to neutralize the ammonia thus formed.

For $\text{C}_4\text{H}_4\text{N}_2\text{O}_2$:

Calculated:
N = 25.05 per cent.

Found:
24.94 per cent.

The mother liquid of the uracil was neutralized with barium hydrate, filtered, and from the filtrate cytosin precipitated by means of a concentrated solution of picric acid. The two picric acid precipitates were combined and transformed into the sulphate in the usual manner. Typical crystals of the basic salt of cytosin appeared. This sulphate was then transformed into the platinic chloride double salt, and as such analyzed.

It had the following composition :

0.144 gm. of the substance gave 0.0440 gm. of Pt.

For $(C_4H_6N_3O)_2$, $PtCl_4$, $2HCl$:

Calculated :
Pt = 30.87 per cent.

Found :
30.55 per cent.

The mother liquid of the cytosin picrate was treated with sulphuric acid and ether to remove picric acid, and then with phosphotungstic acid to precipitate hystidin. The phosphotungstic precipitate was decomposed in the usual manner, and the solution obtained in this way evaporated to dryness under diminished pressure. The residue was dissolved in strong hydrochloric acid, and placed over sulphuric acid in a vacuum desiccator. Hystidin, however, did not crystallize. The solution was then taken up with absolute alcohol, and allowed to stand, and crystalline precipitate of the hystidin hydrochloride then was formed. The yield, however, was quite small.

In the second experiment the gland was taken up in 0.5 per cent sodium carbonate solution, and subjected to autolysis for two months. At the end of that time the sodium carbonate was neutralized with acetic acid, and the product of autolysis concentrated until the weight of the mass was reduced to the original five pounds. It was then treated like the gland in the previous experiment.

Only uracil was obtained. The substance employed for analysis had the following composition :

0.1420 gm. of the substance was employed for a Kjeldahl nitrogen estimation. The Gunning modification was used. It required 25.2 c.c. of $\frac{N}{10}$ H_2SO_4 for neutralization.

For $C_4H_4N_2O_2$:

Calculated :
N = 25.05 per cent.

Found :
24.84 per cent.

The neutralized mother liquid from uracil gave no precipitate with a concentrated solution of picric acid, thus showing the absence of cytosin. The picric acid was then removed in the usual manner and the solution thus obtained was treated with a solution of phosphotungstic acid to remove hystidin. A very small precipitate resulted. The precipitate was decomposed in the usual manner. The yield of hystidin from this precipitate was very insignificant.

The comparative yields of the bases are shown in the following table. 5 lbs. of the gland were used in such experiment.

	Fresh gland:	Fermented gland:
Uracil:	0.460 gm.	0.600 gm.
Cytosin:	1.0 "	0.0 "
Hystidin:	trace	trace

Hexon bases. — Arginin and lysin were found by Kutscher among the products of self-digestion of the gland. The two bases were found also on acid hydrolysis of the gland. However, on acid hydrolysis of the fermented gland neither of these bases could be detected. On their place tetramethylene-diamin was identified. An analogous observation was made by Lawrow. Among the end-products of self-digestion of the gastric wall he failed to discover any arginin or lysin, but did find tetramethylene-diamin and pentamethylene-diamin. The difference in the observations of Kutscher and those of the author again may be explained by the fact that intracellular enzymes cannot be easily extracted, and that therefore the tissue itself is more active than any extract of it.

Experimental part. — The filtrate from pyrimidin fraction was treated according to Kossel for removing arginin. The substance was identified as its silver nitrate salt.

0.1905 gm. of the substance gave 0.0520 gm. of Ag.

For $C_6H_{14}N_4O_2$, HNO_3 , $AgNO_3$:

Calculated:	Found:
Ag = 26.54 per cent.	26.75 per cent.

The filtrate from arginin served for obtaining lysin. The base was precipitated by means of phosphotungstic acid, the acid removed in the usual manner, and lysin precipitated by means of an alcoholic solution of picric acid. The salt was recrystallized out of water once and dried in toluol bath and analyzed.

0.1282 gm. of the substance gave on combustion 0.1797 gm. of CO_2 and 0.0570 gm. of H_2O .

For $C_6H_{14}N_2O_2$, $C_6H_2(NO_2)_3OH$:

Calculated:	Found:
C = 38.40 per cent.	38.31 per cent.
H = 4.53 "	5.00 "

The digested glands were treated in the same manner, but the attempt to obtain arginin from the corresponding fraction proved futile. The lysin fraction was evaporated to a very small volume. A small part of it tested with an alcoholic solution of picric acid gave no precipitate; it, however, formed a crystalline picrate on the addi-

tion of a concentrated solution of sodium picrate. Washed with alcohol and ether, and dried in toluol bath, it had the following composition:

0.1190 gm. of the substance gave on combustion:

0.1538 gm. CO_2 , 0.0415 gm. of H_2O .

0.1513 gm. of the substance gave on combustion 270 c.c. of nitrogen (over 50 per cent KOH) at $t^\circ = 24.0$ C. and $p = 755$ mm.

For $\text{C}_4\text{H}_{12}\text{N}_2$, $2 \text{C}_6\text{H}_2(\text{NO}_2)_3\text{OH}$:

Calculated:	Found:
C = 35.16 per cent.	35.24 per cent.
H = 3.30 "	3.87 "
N = 20.51 "	20.79 "

The comparative yields of the bases are shown in the following table. Five pounds of the gland were used in each experiment.

	Fresh gland:	Fermented gland:
Arginin:	6.4 gms.	0.0 gm.
Lysin:	4.8 "	0.0 "
Putrescin:	0.0 "	0.5 "

AMMONIA.

It was found that the fermented glands yield on hydrolysis with hydrochloric more ammonia than the fresh ones.

Experimental part. — The hydrochloric acid solution of the glands used for the estimation of the pyrimidin and hexon bases was concentrated to a definite volume. 10 c.c. of the solution were diluted to 50 c.c. 5 c.c. of this solution were used for a Kjeldahl nitrogen estimation. 20 c.c. of the same solution were partly neutralized with a solution of caustic potash, then rendered alkaline with magnesium oxide and distilled under diminished pressure at 40° C. until the distillate ceased to contain ammonia.

The comparative yields calculated for five pounds of the glands were as follows:

	Fresh gland:	Fermented gland:
Total nitrogen:	48.44 gms.	57.68 gms.
Nitrogen as ammonia:	6.06 "	10.06 "
Nitrogen ammonia in percentage of the total:	12.34 per cent.	17.44 per cent.

SPLEEN.

Purin bases.—As stated already, while this investigation was in progress the very interesting work of Jones appeared. Jones came to the conclusion that on self-digestion of the spleen guanin remains unaltered, adenin is transformed into hypoxanthin, and that no xanthin can be detected. The digestion was continued five days (only the soluble part of the gland being used for experiment),—and during that time no diminution in the total quantity of purin bases was observed. In the present investigation the entire gland was used for the experiment, and the digestion lasted in one experiment twelve, and in the other fifteen weeks. The results were very similar to those obtained on digestion of the pancreas. The total amount of purin bases was greatly diminished in the digested organs. Guanin and adenin apparently disappeared, while hypoxanthin was isolated in a considerable quantity and xanthin in a very small quantity. However, the fresh glands contained besides guanin and adenin, also hypoxanthin and xanthin, and the quantity of hypoxanthin was not much lower than in the digested gland. Still the assumption that hypoxanthin and xanthin are intermediate products seems quite justifiable, especially in view of the observation of Jones, and those of the writer regarding the pancreas. The source of the hypoxanthin and xanthin in the fresh gland still remains to be ascertained. The nucleic acid obtained by the writer's second process does not seem to contain any of these two bases; but whether they are derived from some other nucleic acid not yet isolated, or whether they are oxidation products of adenin and guanin, will be the subject of a special investigation.

Experimental part.—10 lbs. of the gland were prepared in the usual manner and decomposed with a 5 per cent solution of sulphuric acid. The further treatment was the same as that described in the pancreas experiment. The crude guanin was dissolved in dilute sulphuric acid, decolorized with charcoal, and filtered. The sulphate was converted into the free base, and this into the sulphate. For analysis, the sulphate again was transformed into the free base. It was washed with alcohol and ether, and dried in xylol bath.

0.1122 gm. of the substance gave 45 c.c. of nitrogen (over 50 per cent KOH) at $t^{\circ} = 23^{\circ}$ C. and $p = 765$ mm.

For $C_5H_5N_5O$:

Calculated:	Found:
N = 46.36 per cent.	46.68 per cent.

The mother liquid from guanin sulphate was rendered alkaline by ammonia, then acidulated with acetic acid, boiled, and filtered. The clear filtrate was allowed to cool. The precipitate this formed was washed with alcohol and ether, dried in xylol bath, and analyzed.

0.1130 gm. of the substance was employed for a Kjeldahl nitrogen estimation. 30.20 $\frac{N}{10}$ H_2SO_4 were required to neutralize the ammonia thus formed.

For $C_5H_4N_4O_2$:

For $C_5H_6N_4O_2$:

Calculated:	Found:
N = 36.84 per cent.	33.70 per cent.
	37.36 per cent.

The substance thus could be either xanthin or a mixture of methylxanthin with guanin. Part of it was then dissolved in a normal sodium hydrate solution, and an excess of nitric acid (two parts of strong acid to three parts of water) was added. On standing, a crystalline deposit formed, which showed that the substance was xanthin.

The filtrate from crude guanin was treated with ammoniacal silver chloride, and the silver salts thus obtained were decomposed with hydrochloric acid. The hot filtrate from silver chloride was treated with a solution of sodium picrate, and the adenin picrate was removed on suction funnel while the mother liquid was warm.

After it was once recrystallized out of water, washed with alcohol and ether, and dried in toluol bath, the substance had the following composition:

0.1330 gm. gave on combustion 0.1755 gm. of CO_2 and 0.0315 gm. of H_2O .

For $C_5H_6N_5C_6H_2(NO_2)_3OH$:

Calculated:	Found:
C = 36.26 per cent.	35.99 per cent.
H = 2.20 "	2.63 "

The filtrate from adenin picrate gave on concentration another precipitate. To this precipitate sufficient boiling water was added to effect solution. On standing, the solution turned into a crystalline mass, consisting of very long (some reaching 2 cm.), light yellow crystals. They were filtered on suction funnel, washed with alcohol and ether, dried in xylol bath and analyzed.

0.1630 gm. of the substance gave on combustion 33.5 c.c. of nitrogen (over 50 per cent KOH) at $t^{\circ} = 24^{\circ}$ C. and $p = 760$ mm.

The substance was recrystallized and analyzed.

0.135 gm. of the substance gave on combustion 28.0 c.c. of nitrogen (over 50 per cent KOH) at $t^{\circ} = 25^{\circ}$ C. and $p = 760$ mm.

For $C_5H_4N_4O$, $C_6H_2(NO_2)_3OH$: For $C_7H_8N_4O$, $C_6H_2(NO_2)_3OH$:

	Calculated:	Found:
N = 26.85 per cent.	23.96 per cent.	23.72 per cent.
		23.86 "

Whether this substance was an impure hypoxanthine or paraxanthine will be established by further experiment. There is not sufficient material to do so at present.

The filtrate from this precipitate was acidulated by hydrochloric acid and extracted with ether. The solution free from picric acid was rendered ammoniacal and treated with an ammoniacal solution of silver chloride, and the precipitate thus formed filtered on suction funnel, and decomposed with hydrochloric acid. The filtrate from silver chloride was concentrated to dryness under diminished pressure, the residue taken up in warm water and again concentrated to dryness. This operation was repeated several times, and finally the residue was taken up in alcohol and evaporated to dryness under diminished pressure. The water insoluble part of the residue was dissolved in normal sodium hydrate solution and to the solution an excess of nitric acid (two parts of strong nitric to three parts of water) was added. On standing, a crystalline sediment formed. It gave a very pronounced "xanthin" reaction, and thus may be regarded as xanthin.

The water soluble part of the hydrochloric acid residue was treated with sodium picrate. The precipitate was dissolved in hot water. On cooling, a precipitate formed, which was removed by filtration. It consisted apparently of adenin picrate, and on standing, a second precipitate of hypoxanthin picrate appears. Washed and dried in the usual manner, it had the following composition:

0.1820 gm. of the substance gave 42 c.c. of nitrogen (over 50 per cent KOH) at $t^{\circ} = 22.5^{\circ}$ C. and $p = 762$ mm.

For $C_5H_4N_4O$, $C_6H_2(NO_2)_3OH$:

	Calculated:	Found:
N = 26.85 per cent.		26.86 per cent.

Digested glands.—5 lbs. of spleen were prepared in the usual manner and allowed to digest in 0.2 per cent solution of acetic

acid for twelve weeks. The products of digestion were concentrated to the original weight and decomposed with a 5 per cent solution of sulphuric acid. The product was treated in the usual manner. Neither guanin nor adenin picrate was obtained. In place of adenin picrate, on standing forty-eight hours a picrate appeared resembling that of hypoxanthin. It was recrystallized out of water, washed with alcohol and ether, dried in toluol bath, and analyzed.

0.148 gm. of the substance gave on combustion 34.0 c.c. nitrogen (over 50 per cent KOH) at $t^{\circ} = 26.0^{\circ}$ C. and $p = 762$.

For $C_5H_4N_4O$, $C_6H_2(NO_2)_3OH$:

Calculated:	Found:
N = 26.85 per cent.	26.30 per cent.

The filtrate from this precipitate was acidulated with hydrochloric acid, extracted with ether, rendered ammoniacal, and then treated with an ammoniacal solution of silver chloride.

The precipitate thus obtained was very small. Further separation of the bases was accomplished by Kossel's method.

The silver salt insoluble in nitric acid of sp. gr. 1.1 had the following composition:

0.1510 gm. of the substance gave 0.0526 gm. of Ag.

For $C_5H_4N_4O$. $AgNO_3$:

Calculated:	Found:
Ag = 35.29 per cent.	34.83 per cent.

The filtrate from this precipitate gave on neutralization with ammonia only a very small flocculent precipitate, which might have been xanthin.

In the second experiment 15 lbs. of the gland were allowed to digest fifteen weeks. They were treated in the same manner as in the first experiment, and the results were practically the same. Only a very small precipitate appeared in place of crude guanin, so that it was considered useless to purify. No adenin picrate was obtained at all. Hypoxanthin was removed as a picrate, the mother liquid of this salt was treated with hydrochloric acid and ether to remove picric acid, and the remaining purin bases were removed by an ammoniacal solution of silver chloride. The precipitate thus obtained was decomposed by hydrochloric acid and the filtrate from silver chloride was concentrated under diminished pressure. The residue was treated as described in previous experiments to remove the excess of hydro-

chloric acid. The water insoluble part was dissolved in a normal sodium hydrate solution and an excess of nitric acid (two parts of acid to three parts of water). On standing a crystalline deposit, giving a pronounced xanthin test, appeared. This substance, therefore, might have been xanthin.

The comparative yields, calculated for 5 lbs. of the gland, were as follows:

	Fresh gland:	Digested gland:
Adenin:	1.85 gms.	0.0 gm.
Guanin:	1.10 "	0.0 "
Hypoxanthin:	0.50 "	1.2 "
Xanthin:	0.40 "	0.150 "

Pyrimidin bases.—Concerning the pyrimidin bases of the gland it is known from the writer's analysis of the spleen-nucleic acid, that all three bases occur, and that thymin predominates over the other two. Jones, who recently analyzed the pyrimidin bases of the self-digested spleen, detected only uracil.

In the present investigation, thymin and cytosin were obtained from the fresh gland, and thymin and uracil from the digested gland. Thus the assumption of Jones that cytosin on digestion of the gland is transformed into uracil seems justifiable.

Experimental part.—The procedure in obtaining the pyrimidin bases was identical with that described in the pancreas experiment. The crude bases were dissolved in a 2 per cent solution of sulphuric acid and decolorized with charcoal. The first base to crystallize had the typical appearance of thymin, and the following composition:

0.1300 gm. of the substance was employed for a Kjeldahl nitrogen estimation. 20.5 c.c. $\frac{N}{16}$ H_2SO_4 were required to neutralize the ammonia thus formed.

For $C_5H_6N_2O_2$:

Calculated:	Found:
N = 22.22 per cent.	22.07 per cent.

From the mother liquid of thymin, cytosin picrate was obtained in the usual manner. It had the following composition:

0.1462 gm. of the substance gave on combustion 0.1880 gm. CO_2 and 0.0310 gm. H_2O .

For $C_4H_5N_3O$, $C_6H_2(NO_2)_3OH$:

Calculated:	Found:
C = 35.29 per cent.	35.07 per cent.
H = 2.35 "	2.36 "

From the mother liquid of thymin and cytosin, histidin dichloride was obtained in the same manner as in the pancreas experiments. The yield was very small.

Digested glands were treated in the manner described in the pancreas experiment. The crude bases were dissolved in a hot 2 per cent solution of sulphuric acid, decolorized and allowed to cool. The substance that crystallized first had the appearance and the composition of thymin.

0.1240 gm. of the substance was used for a Kjeldahl nitrogen estimation. 20.10 c.c. $\frac{N}{10}$ H_2SO_4 were required to neutralize the ammonia thus formed.

For $C_5H_6N_2O_2$:

Calculated:	Found:
N = 22.22 per cent.	22.69 per cent.

The mother liquid on standing in vacuum desiccator gave another precipitate resembling uracil. It was twice recrystallized out of 1 per cent solution of sulphuric acid. For analysis it was dried in xylol bath. Its composition was as follows:

0.1285 gm. of the substance was employed for a Kjeldahl nitrogen estimation. 23.30 c.c. of $\frac{N}{10}$ H_2SO_4 were required.

For $C_4H_4N_2O_2$:

Calculated:	Found:
N = 25.05 per cent.	25.38 per cent.

No appreciable quantity of cytosin picrate could be isolated.

The phosphotungstic precipitate supposed to contain histidin was so small that further treatment was abandoned.

The comparative yields of the bases calculated for 5 lbs. of the gland were as follows:

	Fresh gland:	Digested gland:
Thymin:	0.400 gm.	0.380 gm.
Uracil:	0.450 "
Cytosin:	0.300 "	0.0 "
Histidin:	trace	trace

Hexon bases. — Both hexon bases, arginin and lysin, were obtained on acid hydrolysis of the fresh as well as of the digested glands. However, the yields were markedly smaller in the digested glands.

The process employed for obtaining the bases were the same as described in the pancreas experiment.

Arginin was identified as silver nitrate. The analysis of the silver nitrate obtained from the fresh glands gave the following results:

0.1990 gm. of the substance gave 0.535 Ag.

The analysis of the substance obtained from the digested glands gave the following results:

0.2200 gm. of the substance gave 0.0595 of Ag.

For $C_6H_{14}N_4O_2$, HNO_3 , and $AgNO_3$:

Calculated:	Fresh gland:	Digested gland:
Ag = 26.54 per cent.	26.83 per cent.	27.07 per cent.

Lysin was identified as its picrate.

0.1540 gm. of the substance obtained from the fresh gland gave on combustion 0.2170 gm. CO_2 and 0.0700 gm. H_2O .

For $C_6H_{14}N_2O_2$, $C_6H_2(NO_2)_3OH$:

Calculated:	Found:
C = 38.40 per cent.	38.46 per cent.
H = 4.53 "	5.03 "

0.1590 gm. of the substance obtained from the digested glands gave 26.5 c.c. nitrogen (over 50 per cent KOH) at $t^\circ = 26.6^\circ$ C. and p. = 755 mm.

For $C_6H_{14}N_2O_2$, $C_6H_2(NO_2)_3OH$:

Calculated:	Found:
N = 18.67 per cent.	18.92 per cent.

The comparative yields calculated for 5 pounds of the gland were as follows:

	Fresh gland:	Digested gland:
Arginin:	3.2 gms.	1.5 gms.
Lysin:	2.0 "	1.2 "

LIVER.

Purin bases.—In a general way the purin bases undergo the same changes on digestion that they do in the organs. The difference may be only quantitative. Thus, a diminution in the total quantity of bases was noted. Guanin was isolated from the digested gland in a very small quantity, and adenin was obtained, though in comparatively small quantity. In this respect the process in the liver differs from that in the pancreas and spleen. After ten weeks of digestion of

pancreas, adenin could not be detected. Hypoxanthin was isolated from the fresh and from the digested gland, while xanthin could be demonstrated in very small quantity in the fresh gland.

Experimental part. — 10 lbs. of liver were treated in exactly the same manner as described in the experiments on the spleen.

Guanin was analyzed in form of sulphate.

0.1230 gm. of the substance was employed for a Kjeldahl nitrogen estimation. 28.0 c.c. of $\frac{N}{10}$ H_2SO_4 were required.

For $(C_5H_5N_5O)_2H_2SO_4 + 2 H_2O$:

Calculated:	Found:
N = 32.21 per cent.	31.88 per cent.

Adenin was identified as the picrate.

0.1325 gm. of the substance gave 36 c.c. nitrogen (over 50 per cent KOH) at $t^\circ = 26^\circ C.$ and $p = 755$ mm.

For $C_5H_5N_5CH_2(NO_2)_3OH$:

Calculated:	Found:
N = 30.71 per cent.	30.95 per cent.

Hypoxanthin was isolated in the form of the picrate.

0.140 gm. of the substance gave on combustion 33 c.c. nitrogen (over 50 per cent KOH) at $t^\circ = 27^\circ C.$ and $p = 760$ mm.

For $C_5H_4N_4O, C_6H_2(NO_2)_3OH$:

Calculated:	Found:
N = 26.85 per cent.	26.90 per cent.

The picrate was decomposed by nitric acid and ether, and on concentration the nitrate of hypoxanthin crystallized. Xanthin was isolated in a small quantity in form of its insoluble nitrate.

Digested glands. — 5 lbs. of the gland were employed for the experiment. The yield of purin bases was very small.

The crude guanin was once crystallized out of sulphuric acid and then converted into the free base. On analysis it gave the following results:

0.1200 gm. of the substance was employed for a Kjeldahl nitrogen estimation. 33.90 c.c. of $\frac{N}{10}$ H_2SO_4 were required.

For $C_5H_5N_5O$: For $C_5H_4N_4O_2$:

Calculated:	Calculated:	Found:
N = 46.36 per cent.	36.84 per cent.	37.6 per cent.

Thus showing that the substance consisted chiefly of xanthin.

From the filtrate of the guanine fraction adenine and hypoxanthine were isolated in form of picrates. Both were in small quantities. The adenine picrate was once recrystallized and had a MP. = 282° C.

Hypoxanthine gave on analysis the following results:

0.113 gm. of the substance gave 28 c.c. nitrogen (over 50 per cent KOH) at $t^{\circ}=26.5^{\circ}$ C. $p=758$ mm.

For $C_5H_4N_4O$, $C_6H_2(NO_2)_3OH$:

Calculated:	Found:
N = 26.85 per cent.	27.32 per cent.

The comparative yields calculated for 5 lbs. of the glands were as follows:

	Fresh gland:	Digested gland:
Guanine:	0.755 gms.	trace
Adenine:	1.5 "	0.37 gm. (of the crude subst.)
Hypoxanthine:	1.1 "	0.300 "
Xanthine:	very small quantity	0.300 "

Pyrimidine bases.—The yield of these bases was very small. However, a precipitate consisting of the scales typical of thymine was obtained from the fresh gland. From the mother liquid little of cytosine picrate was isolated. It had a melting point of 275° C. Attempts to isolate the bases from the digested gland were futile.

Hexon bases.—Both bases were obtained from the fresh and digested gland. However, the yield from the digested glands was smaller, thus showing that also in the liver the hexon bases undergo further decomposition. The discovery of arginase by Kossel explains the diminution in the yield of arginine.

Arginine was identified in the form of its silver nitrate.

0.1875 gm. of the substance obtained from the fresh gland gave 0.052 gm. of Ag.

0.2460 gm. of the substance obtained from the digested gland gave 0.0655 gm. Ag.

For $C_6H_{14}N_4O_2$, $HNO_3 + AgNO_3$:

Calculated:	Found:	
	Fresh gland:	Digested gland:
Ag = 26.54 per cent.	27.12 per cent.	26.66 per cent.

Lysine was identified in the form of its picrate.

0.1330 gm. of the substance obtained from the fresh gland gave 22.0 c.c. nitrogen (over 50 per cent KOH) at $t^{\circ}=26.5^{\circ}$ C. and $p=760$ mm.

0.2020 gm. of the substance obtained from the digested gland gave 33.0 c.c. of nitrogen (over 50 per cent KOH) at $t^{\circ} = 23^{\circ}$ C. and $p = 762$ mm.

For $C_6H_{14}N_2O_2$, $C_6H_2(NO_2)_3OH$:

Calculated:	Found:	
	Fresh gland:	Digested gland:
N = 18.67 per cent.	18.94 per cent.	19.09 per cent.

The comparative yield calculated for 5 lbs. of the gland were as follows:

	Fresh gland:	Digested gland:
Arginin:	10.8 gms.	5.2 gms.
Lysin:	12.96 "	4.0 "

I wish to express my indebtedness to Prof. R. H. Chittenden, under whose control this investigation was carried out.

The expenses of the work were defrayed by a grant from the Carnegie Institution of Washington.

ON THE SWELLING OF ORGANIC TISSUES.—
RESEARCHES ON THE CORNEA.

By G. BULLOT.

TH. LEBER, in 1873, found that, when the endothelium covering the posterior surface of the cornea is removed, the eyeball being kept entire, the corneal stroma swells considerably and becomes opaque. The swelling is due to the corneal stroma absorbing the aqueous humor filling the anterior chamber of the eye. Normally, this imbibition cannot take place on account of the impermeability of the living endothelium to aqueous humor. Similarly to the corneal endothelium, the epithelium covering the anterior surface of the cornea is also impermeable to water, as Th. Leber and others have shown. But its removal is not followed by any absorption of liquid by the stroma. The cornea of the entire eyeball, with its epithelium scraped off, remains as thin and transparent as it is normally, whether the eyeball is left in situ with the eyelids open or sutured, or is enucleated and transplanted into the peritoneal cavity. (L. Lor and G. Bullot.) The present paper discusses the reason for this difference in the reaction.

I. The phenomenon is not due to a difference in the power of imbibition of the conjunctival layers on each side of the stroma. When the cornea of the rabbit is detached and placed in distilled water, fresh water, or sodium chloride solutions, it swells considerably, whether only the anterior surface or the posterior surface alone has been scraped.

II. It is not due to the quantitative difference which exists in the salt contents of the aqueous humor on one side, and the tears, conjunctival secretion, or peritoneal liquid on the other. S. Ringer, Fr. Hofmeister, J. Loeb pointed out that the degree of swelling of organic tissues or substances is affected by the presence of salts. S. Ringer particularly observed that the dry tissue of the marine alga *Laminaria* swells much more in sodium chloride solutions than in calcium chloride solutions. Now, from what is known of the physiological antagonistic action of sodium and calcium salts, the question

suggests itself whether a similar antagonism exists here in the mere physical process of swelling when those salts are brought together in the same liquid as is the case for organic liquids. The antagonistic action would be obtained, as usual, when a certain relation exists between the quantities of the salts. On the other hand, it is known that the aqueous humor has not the same quantitative salt composition as the other liquids under consideration. It might therefore be that the aqueous humor contains too little calcium salt to prevent the swelling due to sodium chloride, while the tears, conjunctival secretion, and peritoneal liquid possess a sufficient quantity of calcium salts to check the swelling. Such, however, is not the case. At concentrations equal to those of the sodium and calcium salts in organic liquids, or at higher or lower concentrations, there is, as a rule, no antagonism between calcium and sodium, although, at concentrations of the same order of magnitude as the one at which they exist in organic liquids, the calcium salts, when present alone, make the cornea swell much less than the sodium salts alone. Many experiments made either on fresh pieces of the detached cornea or on small strips cut from the dry cornea give the same negative result. Only when the quantity of sodium chloride is very small can its action on the swelling be checked by the calcium salts.

Data.— If pieces of the scraped fresh cornea be placed in various liquids, at a temperature of from 15 to 25° C., it will be observed that the swelling is greatest in distilled water. The thickness attained reaches 3 mm., which is ten times greater than the thickness of the normal cornea.

In sodium chloride solutions from $\frac{m}{10000}$ to 2 *m* the degree of swelling is distinctly less.¹ The thickness of about 2 mm., in $\frac{m}{10000}$, decreases very slowly with an increase in the concentration of sodium chloride so that, in 2 *m* sodium chloride, it is equal to 1.5 mm. (Identical results are obtained with solutions of potassium chloride, lithium chloride, ammonium chloride, sodium bromide, sodium iodide, sodium fluoride, sodium nitrate, sodium sulphate, sodium carbonate.)²

¹ This behavior of the cornea differs from that of gelatine, which was found by Fr. Hofmeister to swell more in sodium chloride solutions than in distilled water.

² To test the action of all these salts, only narrow strips cut from the dry stretched cornea were employed. Their use is to be desired, on account of the quickness of the swelling and the number of trials which can be performed with a single cornea. Such pieces cannot always be used because the degree of swelling is less than with pieces of the fresh cornea (2 mm. in distilled water). The same relation exists, however, between the degree of swelling in distilled water and in the various sodium chloride solutions.

With calcium chloride solutions from $\frac{m}{10000}$ to m , it is observed that the cornea swells the least in $\frac{m}{100}$; the swelling is much less than in any of the sodium chloride solutions. With higher or lower concentrations, the degree of swelling gradually increases, but the increase is greater towards m than towards $\frac{m}{10000}$. (Exactly similar results are reached with barium chloride, strontium chloride, and magnesium chloride solutions, with regard to the shape of the curve plotted with the figures of the concentration, and the degree of swelling, and with regard to the absolute degree of the swelling.)¹

In mixtures of sodium chloride and calcium chloride an antagonistic action of calcium chloride may be clearly seen only when the composition is about $\frac{m}{100}$ NaCl and $\frac{m}{100}$ CaCl₂. In this case, the degree of swelling is as small as in solutions of calcium chloride alone. When the mixture contains $\frac{m}{10}$ NaCl, which is about the concentration of sodium chloride in the organic liquids, the antagonistic action of calcium chloride completely disappears.

III. The difference we are discussing is due principally to the action of the intraocular pressure which does not prevent imbibition through the posterior surface, both forces acting in the same direction, but which opposes imbibition through the anterior surface. When the entire eyeballs of the rabbit, with the epithelium scraped off, are suspended in various solutions of sodium chloride from $\frac{2}{100} m$ to $\frac{16}{100} m$, it is observed, after a certain number of hours that those solutions which produce hypertension of the eyeball, on account of their hypotonicity compared with the internal liquids of the eye, leave the cornea thin and nearly transparent. On the contrary, in the hypertonic solutions, *i. e.* solutions above $\frac{12}{100} m$, in which a hypotension of the eyeball is determined, the cornea swells considerably. This result cannot be attributed to the direct action of the solution on the cornea since we saw, in the case of the detached cornea, that the swelling is considerable in all concentrations of sodium chloride and less in the high concentrations than in the low ones. In distilled water, in which a very strong hypertension is produced, the cornea after a certain number of hours is as thin as in the normal state. Later, however, when through the injurious action of the distilled water, the endothelium of the posterior surface of the cornea is killed, the cornea swells through imbibition of aqueous humor. In fresh water, which has no such injurious action on the endothelium, and in which also a strong hypertension is pro-

¹ Also studied with strips of the dry cornea.

duced, the cornea remains of normal thinness even after two days. Results quite similar to those obtained with sodium chloride are reached with various concentrations of cane-sugar, which has proved to be of no influence on the swelling of the detached cornea. (Whatever may be the concentration of cane-sugar from $\frac{m}{10}$ to m , the degree of swelling of the detached cornea is identical with that in distilled water.)

On the normal eyeball in situ in the living animal, the intraocular pressure is quite sufficient to prevent the imbibition of the scraped stroma through the anterior surface. The same is true when the enucleated eyeball is placed in the peritoneal cavity of the living animal. But when the enucleated eyeball is suspended in blood serum or white of egg, the imbibition through the anterior surface is also checked, at least partially, in spite of a decrease in the intraocular pressure, which is readily determined under those conditions. Here a second factor must be considered.

IV. This second factor which, in case the eye has been enucleated and placed in albuminoid liquids, intervenes independently of the intraocular pressure to produce the difference in the degree of imbibition of the corneal stroma through the anterior and posterior surface, is the action of colloidal substances present in albuminoid liquids and nearly completely absent in the aqueous humor. E. H. Starling has found, using blood serum, that the albuminoid substances of blood serum have an osmotic pressure of about 35 mm. Hg. As there is about seven per cent of albuminoid substances in blood serum, the pressure would be about 5 mm. Hg for each per cent. Therefore, it is to be expected that when blood serum is brought into contact with a tissue capable of absorbing water, it will give water to that tissue only if the force of imbibition which causes the water to go into the absorbing tissue is greater than the osmotic pressure of the serum. And conversely, when the tissue is already completely swelled, the blood serum will absorb water from it and diminish the degree of swelling if the osmotic pressure is greater than the force which holds the water in the swollen tissue.

The first point is proved by the fact that, when the scraped eyeball is placed in blood serum, although the intraocular pressure decreases, the cornea swells very slowly and very moderately (1 mm.), much less than if it had been placed in a sodium chloride solution possessing the same decreasing effect on the intraocular pressure. This cannot be due to the action of the calcium salts of the serum, since it

has been said above that, at the concentration at which sodium chloride exists in blood serum, the calcium salts have no longer any antagonistic action on them, as far as the process of swelling is concerned. When white of egg is used instead of blood serum, similar results are obtained, but the degree of swelling is still less. With blood serum as well as with white of egg, the cornea, although swollen, remains transparent. Such a state of transparency is permanent in blood serum. In white of egg, it is followed, after a certain number of hours, by the appearance of a moderate opacity which is not accompanied by any further increase in the degree of swelling. This leads to the conclusion that the albuminoid solution goes into the corneal stroma, since, in salt solutions free from colloidal substances, the swelling is always accompanied by opacity except in case of sufficiently concentrated solutions of salts of bivalent metals. If, instead of the entire eyeball, pieces of the scraped cornea are placed in blood serum or white of egg they swell, but much less than in equivalent solutions of sodium chloride (1 mm.) and less in white of egg than in blood serum.

The second point, the subtraction of water by albuminoid solutions from the already swollen cornea is made evident in the following manner. Three entire eyeballs, with the corneal epithelium scraped off, are first placed in a $\frac{1}{2}$ *m* solution of cane-sugar, which is hypertonic compared with the liquids of the eye and the albuminoid solutions to be used afterwards. After two or three hours the corneas are completely opaque; the hypotension of the eyeballs is considerable. From one of them, the cornea is then detached and is found to be strongly swollen with a liquid rich in cane-sugar. This gives an indication concerning the degree of swelling of the cornea of the two other eyeballs at that moment. One of them is then transferred into blood serum or white of egg. After three or four hours in case of white of egg, five or six hours in case of blood serum, the cornea becomes again completely transparent and, being detached, shows itself to be again nearly as thin as normal. The cornea of the third eyeball which was kept in cane-sugar being now detached, shows the same degree of opacity and of swelling as the first one. This proves that white of egg and blood serum have the power of subtracting water from the swollen cornea, even when the liquid of imbibition is hypertonic compared with those colloidal liquids. In case the second eyeball should have been kept longer in white of egg or blood serum, the cornea would again have swelled moderately,

more in blood serum than in white of egg, keeping its transparency in blood serum, becoming gradually opaque in white of egg, so that identically the same state of affairs would exist as when the eye is directly placed in serum or white of egg without passing through cane-sugar. Here, as above, the state of permanent transparency of the cornea in the case where blood serum is used, warrants the conclusion that some of the colloidal substance has entered this membrane.

The first subtraction of water from the swollen cornea, under the influence of albuminoid liquids, cannot be attributed to the osmotic action of the salts of the blood serum, as the cornea has imbibed a hypertonic solution. Nor could this be due to inequality in the initial rate of osmosis of cane-sugar and of the salts of the blood which, after a certain time, would make the blood serum more concentrated than the liquid imbibing the stroma and enable it to absorb water from the cornea through osmosis (Lazarus Barlow). It could not be so, because when the swollen cornea is placed in any concentration of sodium chloride or calcium chloride, even if the imbibing liquid is distilled water, the swelling diminishes only to a small extent. It is therefore necessary to attribute the subtraction of water to the action of the colloidal substance of those two liquids.

If, instead of the entire eyeball, pieces of the detached scraped cornea are placed first in $\frac{1}{2} m$ cane-sugar and, after swelling, in white of egg or blood serum, a similar subtraction of water is observed. When the fragments are again placed in cane-sugar, the cornea again swells, to decrease in thickness a second time when replaced in white of egg, and this to the same extent as before. The subtraction of water from a hypertonic salt solution through a permeable membrane by a colloidal solution has long been known. Lately E. H. Starling attributed it to the osmotic pressure of the colloidal substance which is compelled to remain on one side of the membrane, while for the salts, as they can pass through the membrane, an equilibrium is soon reached on both sides. Thus the colloidal substance, by a weak but continuous action, gradually subtracts the water placed on the other side. The same occurs here, with this only difference that, instead of the mere hypertonic crystalloid solution, a swollen tissue imbibed with the solution is present. After a certain time, the colloidal substance penetrating slowly into the cornea, a certain degree of swelling is again possible.

The subtraction of water from the previously swollen corneal stroma by an albuminoid liquid suggests the question of the struggle for water between colloids of different kinds. This question will be studied in a further paper.

My thanks are due to Mr. H. Hus for his valuable assistance.

ON DECAPSULATION OF THE KIDNEY.¹

By ISAAC LEVIN.

THE rôle of the renal capsule for the function of the kidney is scarcely ascertained, less so is its rôle in the economy of the entire organism. There exists also very little knowledge regarding the effect of the removal of the capsule on the function of the kidney. Notwithstanding this, decapsulation of kidneys has been recommended and practised for curative purposes. Thus R. Harrison, J. Israel, and A. Pousson reported cases where they performed nephrotomy on patients suffering from colicky pains, hematuria, and albuminuria, in the expectation of finding tuberculosis, calculus, or pus in the kidney. At the operation neither of these conditions was found, and still the symptoms, including even albuminuria, were cured. In some of these kidneys they discovered various inflammatory processes, and the albumen as well as casts found in the urine before the operation, disappeared after it. All this led the authors to believe that certain cases of nephritis may be improved or even cured by a unilateral nephrotomy. They explain the beneficial effect of the operation by the relief of the organ from congestive swelling and increased tension.

G. Edebohls came to the same conclusion from his experience with a different class of cases. He noticed in his operations for floating kidney, that the albumen and casts sometimes present before the operation, disappeared after it. His operation for floating kidney consists in partial decapsulation and anchoring of the kidney. He then took up cases of simple Bright's disease, decapsulated the kidney, and saw his patients improve or recover. He goes much further in his conclusions than the former authors, and thinks that every case of Bright's disease can be cured by decapsulation of the kidneys. He explains the beneficial effect of his operation by subsequent adhesive inflammation, which establishes a collateral circulation.

¹ Read at the annual meeting of the Association of American Pathologists and Bacteriologists, April 1, 1904.

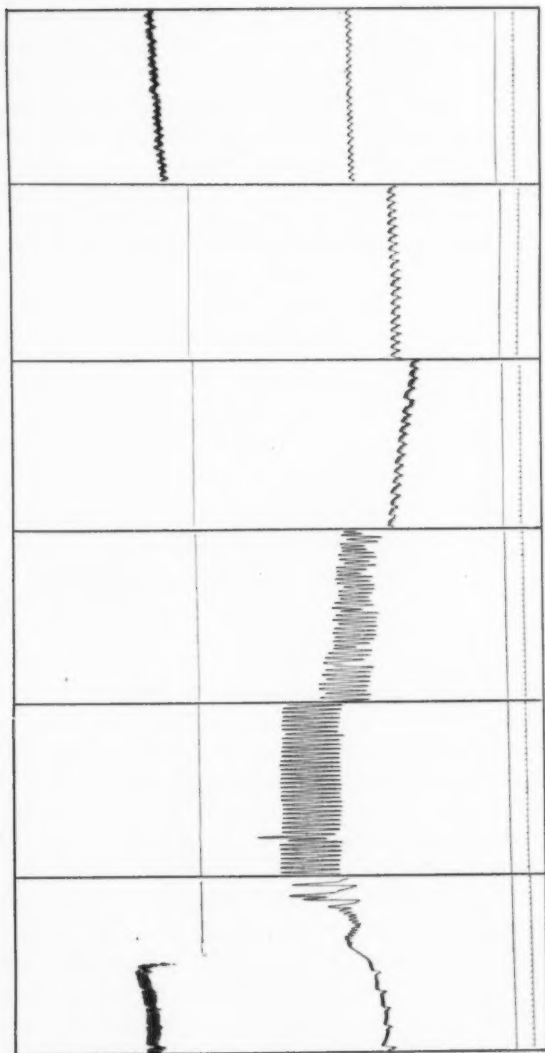


FIGURE 1.—Portions of a prolonged record showing the effect of adrenalin on the kidney. The uppermost tracing is the volume curve of the kidney; the next lower the curve of the blood pressure in the carotid artery. The third line is the atmospheric pressure; the lowest line gives the time in seconds. The 1st, 2d, 3d, and 4th vertical lines indicate an interval of sixty seconds. The records during this period are not reproduced because of lack of space. The 5th vertical line represents an interval of six minutes, thirty seconds.

Decapsulation of the kidney is now the generally accepted operative interference in Bright's disease. We see, then, that a number of modern surgeons consider the removal of the fibrous capsule of the kidney beneficial in certain diseases, while the function of the capsule is practically unknown. The question may evidently arise whether the benefit of the decapsulation, admitting that such actually exists, may not be more than counterbalanced by the injury done to the organism by depriving the kidney of its covering.

I therefore undertook to examine into the possible function of the fibrous capsule of the kidney. If we compare the fibrous coverings of the liver, spleen, pancreas, and all other parenchymatous organs on

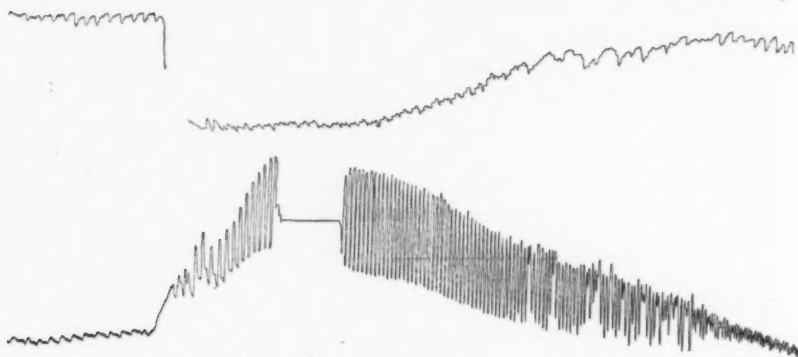


FIGURE 2. — Normal kidney. Adrenalin injection.

one hand, and those of the kidney on the other, we cannot fail to notice a great difference between them. While the former is very thin and firmly adherent, practically an integral part of the organ, the latter is a strong fibrous covering, easily detached from the kidney. After such detachment of the capsule, or decapsulation, the kidney immediately swells. All this makes it already, a priori, very probable that the capsule of the kidney is functionally more important than the capsules of the other parenchymatous organs.

The question arises how to discover the influence of the capsule on the kidney. I chose the oncometric method of investigation, which

records the minutest changes in the size of the kidney; and here I thought, *a priori*, the influence of the capsule would be seen. The oncometer is in principle a plethysmograph especially modified for use upon the kidney. The apparatus which is used in the physiological laboratory of Columbia University is a bivalved, kidney-shaped metal box, hinged at the back, with a clasp in front, while a grooved notch at the centre of the rims of the valves prevents pressure on the vessels and nerves of the pedicle of the kidney when the organ is enclosed in the oncometer. In the interior of the box two pieces of thin soft rubber are so fastened to each half that a layer of water may be placed between them and the metal walls of the box. There is thus formed in each half of the box a soft water-pad, on which the kidney rests. When the kidney, freed from fat and surrounding

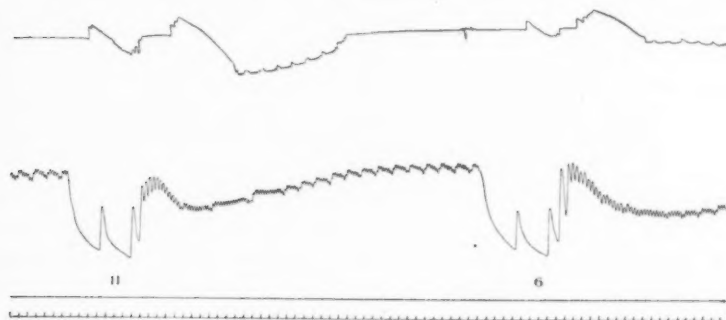


FIGURE 3. — Decapsulated kidney. Vagus stimulated.

connective tissue, but with the blood-vessels and nerves entering at the hilus entirely uninjured, is laid in one half of the oncometer, and the other half is shut down upon it and tightly fastened, then each expansion or contraction of the kidney changes the quantity of water in the upper half of the oncometer. A short metal tube in the centre of the upper valve, to which is attached a piece of soft rubber tubing, allows the water in the upper half of the oncometer to rise or fall in the tube. This movement in the water column is communicated to a column of air farther in the tubing, which in turn communicates with the air of a tambour, which records the oncometric tracing.

The different agents producing a shrinking of the kidney, and consequently a fall of the oncometric tracing, can be divided into two classes. The agents of the first class produce that effect by

actively contracting the blood-vessels of the kidney simultaneously with the rise of the general blood pressure. The most powerful agent of this class is adrenalin. To the second class of agents belongs the stimulation of the vagus nerve. The shrinking of the kidney caused by this stimulation is not due to the contraction of the kidney vessels, but to the diminished supply of blood to the kidney through the weakening of the heart action and consequent fall in general blood pressure. While the agents of the first class produce simultaneously a fall of the kidney tracing, and rise of the carotis tracing, after the stimulation of the vagus both tracings fall. In my experiments I tried both adrenalin and stimulation of the vagus.

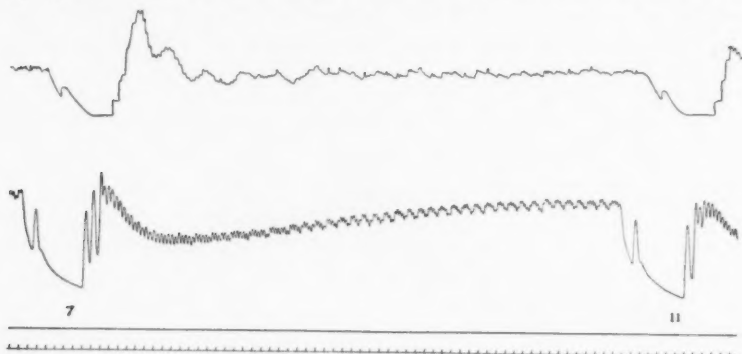


FIGURE 4. — Normal kidney. Vagus stimulated.

The following is the course of my experiments :

One of the kidneys of a dog was decapsulated through the lumbar or abdominal incision. Twenty-four or forty-eight hours later the abdomen of the same dog was opened, one of the kidneys placed in the box and connected with the recording apparatus. The carotis was also connected with a sphygmograph in order to watch the effect of the agents on the general circulation. The normal pulsation was recorded for some time on the kidney and carotis, then either adrenalin injected or the vagus stimulated. When the influence of the agents on both the kidney and carotis disappeared and the pulsation of both returned to the normal, the first kidney was removed from the box, the other placed into it, and the same experiment repeated. I performed this experiment on eighteen dogs with nearly identical results.

Comparing the tracings of the normal kidneys with those of the decapsulated ones, one notices that in the former, immediately after the injection of the adrenalin or the stimulation of the vagus, the tracing falls, then continues for some time on the same level, but always shows pulsation and returns to the old level, mostly even before the tracing of the carotis becomes normal. In the decapsulated kidney the tracing also falls immediately after the injection, then for a considerable length of time continues as a straight line, showing an absolute cessation of pulsation in the kidney, and returns to normal much later than the carotid blood pressure. The analysis of the result of the experiments justifies the following conclusion:

Any stimulus which, either by contracting the general blood pressure or by weakening the action of the heart, diminishes the size of the kidney, exerts a much stronger influence on a decapsulated kidney than on a normal one, and this influence also lasts longer on the former. This fact can be explained by the assumption that the capsule acts like an elastic covering. On one hand it prevents an undue overfilling of the kidney with blood, on the other hand it does not allow the kidney to remain contracted and bloodless for a long time. The other possible explanation, the theory that the decapsulation injures the nervous apparatus of the kidney and thereby changes the action of these agents is not tenable, because the vasomotor nerves of the kidney come from the splanchnics, and the division of the latter (the only effect decapsulation can produce on the nerve) increases the size of the kidney; consequently the action of our agents ought to have been less marked on the decapsulated kidney.

The research is as yet not finished. I expect to repeat it on kidneys with longer periods of decapsulation and on diseased kidneys, but the results already obtained seem to warrant the assumption that the capsule may have a much more serious influence on the function of the kidney in health and disease than one would think a priori; that it may be a powerful protection against noxious agents.

In conclusion I desire to express my gratitude to Professors J. G. Curtis and F. S. Lee, in whose laboratory this work was done.

CHANGES IN THE EXCRETION OF CARBON DIOXIDE RESULTING FROM BICYCLING.

BY G. O. HIGLEY AND W. P. BOWEN.

[*From the Physiological Laboratory of the University of Michigan.*]

I. INTRODUCTION.

IN a series of studies of the effects of muscular work that have been made in this laboratory, the purpose has been to secure results that will be of service in building up a more complete scientific basis for physical education. The value of results for this purpose depends to a large extent upon the completeness with which the effects of the work are recorded. It is not necessary that every study should take account of all or even many of the effects produced by the work, but it is essential that the effects which are chosen as the subjects of investigation shall be followed continuously as long as they last, and recorded with accuracy. Here lies the weakness of much that has been done to discover the influence of muscular work upon the organism. A great number of these studies consist of only two brief tests. Such researches often bring out striking results which serve as valuable hints at the direction in which the truth lies, but their lack of completeness renders them useless as a working basis for the deduction of practical principles. Muscular work must be studied in the thorough manner followed by clinicians in studying disease. Here each change resulting from the disease is carefully traced through its entire course, thus giving an exact picture of the condition of the patient at every stage. Similarly, in order to know how muscular work may be used most efficiently and with least danger, we want not so much a demonstration of the greatness of its maximum effect as a full and definite knowledge of the course of each effect from its first appearance until normal conditions reappear. With this purpose in view, emphasis has been laid in this group of researches upon making each series of records as complete as possible. Whenever it was feasible, continuous graphic records were made, since they give the most complete

and reliable information obtainable. When graphic records could not be made, the observations were taken as frequently as was necessary to determine the general course of the changes in question, and followed clear through as in case of graphic records. We believe that it is only upon the basis of the knowledge gained by a vast amount of such careful and thorough study that the problems of physical education can ultimately be solved.

The idea of securing a continuous record of the output of carbon dioxide was first suggested by a difficulty in explaining the changes in pulse rate resulting from muscular work. With all vigorous work we find, as has been previously stated,¹ two well marked stages of increase in pulse rate, which are often separated by a period of uniform rate. First there is an immediate and rapid rise called the primary rise, and later a more gradual secondary rise. The primary rise, for reasons stated elsewhere,² must be due to nervous regulation of the heart. The secondary rise is not well understood, but is possibly due to waste products. Since carbon dioxide is one of the chief waste products of muscular metabolism, it was thought that a graphic record of the changes in its output, placed alongside of the curve of pulse rate in question, might throw light on the problem, and could not fail to be of interest.

The problem before us, then, was that of determining accurately the changes in excretion of carbon dioxide simultaneously with changes in the rate of the pulse, during successive periods of rest, vigorous muscular work and recovery.

METHODS.

Before going on to devise a method for ourselves we made a careful study of existing respiration methods. The methods already in use may be classed as: (I) Respiratory chamber methods and (II) Mask or mouth-piece methods.

I. Respiratory chamber methods.— These methods are all modifications of that of Regnault and Reiset.³ As originally constructed, this apparatus was intended exclusively for experiments upon small

¹ BOWEN: Contributions to Medical Research, University of Michigan, 1903, pp. 462-493.

² BOWEN: *Ibid.*, p. 475.

³ REGNAULT and REISET: *Annales de chimie et physique*, Paris, 1849, xxvi, p. 299.

animals. It was later improved by Hoppe-Seyler¹ and adapted to scientific experiments upon larger animals, such as sheep and dogs. A little later it was greatly improved by Pettenkofer² and from that time until the present has been extensively used in experiments upon man. It was further improved by Tigerstedt,³ was given a capacity of a hundred cubic metres and has since been employed by Johansson,⁴ Atwater and Rosa,⁵ and others. Some of the good points of this apparatus are as follows: First, it admits of experiments of indefinite length. Second, it admits of making experiments upon eighteen or more persons at once, thus enabling the experimenter to obtain average values. Third, in its most complete form as employed by Atwater, it performs the work both of a respiration apparatus and of a calorimeter, giving results which are comparable in accuracy to those obtained by the use of the combustion calorimeter and the combustion furnace. It is evident, however, that notwithstanding the many good features of this apparatus it is not at all adapted to the solution of such a problem as lay before us, since no known method for the sampling and analysis of the expired air would permit of a sufficiently accurate determination of the amount of carbon dioxide excreted during the sudden changes accompanying the beginning and the ending of vigorous muscular work. Since this research was begun there has appeared a new method by Jacquet.⁶ This is also a respiratory chamber method but the chamber is greatly reduced in size, thus permitting a determination both of the carbon dioxide excreted and of the oxygen absorbed. According to this method, however, the subject is obliged to recline, and the apparatus is therefore quite unsuited to the performance of bicycle work such as was contemplated in our research.

II. Mouth-piece methods.—1. *Method of Speck.*⁷ According to this method the subject, with closed nostrils, breathes through a mouth-piece, air drawn from a calibrated spirometer, the expired air being collected in a second spirometer. At the close of the experiment a

¹ HOPPE-SEYLER: *Archiv für die gesammte Physiologie*, 1876, xxi, p. 18.

² PETTENKOFER: *Annalen der Chemie und Pharmacie*, 1862-63, Supplement Band ii, p. 17.

³ TIGERSTEDT: *Skandinavisches Archiv für Physiologie*, 1895, vi, p. 1.

⁴ JOHANSSON: *Ibid.*, 1901, xi, p. 273.

⁵ ATWATER: *United States Department of Agriculture bulletin*, 63, 1899.

⁶ JACQUET: *Verhandlungen der naturwissenschaftlichen Gesellschaft in Basel*, 1903, xii, p. 18.

⁷ SPECK: *Physiologie des menschlichen Athmens*, 1892, p. 95.

sample of air is drawn from the expiration spirometer and its percentage of carbon dioxide and of oxygen determined by absorption with barium hydroxide and pyrogallol respectively.

This method seemed unsuited to our purpose since (1) the spirometers would need to be inconveniently large in order to contain air necessary for a work experiment lasting an hour or more; and (2) only the total carbon dioxide excreted could be determined while it was necessary for our purpose to determine the character of all changes. Moreover, as it seems to us, respiration with closed nostrils, through a mouth-piece, can scarcely be called normal. Some experiments made by ourselves with one of these mouth-pieces satisfied us that the resulting dryness of the mouth and throat renders the method quite objectionable.

2. *Method of Geppert and Zuntz.*¹ According to this method the expired air is forced through a carefully calibrated gas-meter and its volume accurately measured. Samples of the air are taken by means of a special sampling device which is operated by the gas-meter itself. A number of tubes with capillary at the upper end are filled to the tip with acid water. These are connected to a lowering device which is driven by a belt running over a pulley on the main axis of the gas-meter. As the air passes through the gas-meter the pulley revolves, the levelling tube connected with the collecting apparatus is gradually lowered and the collecting tube is thus filled with air, whose composition has been found to represent quite accurately that of the air passing through the meter. These samples of air are then analyzed for carbon dioxide and oxygen, and there is thus obtained both the carbon dioxide excreted and the oxygen absorbed, giving, of course, the respiratory quotient. This is an excellent method and to it we are indebted for a large amount of our knowledge respecting respiration. However, by this method, it would be necessary either to multiply greatly the number of absorption tubes and thus greatly complicate the gas analysis, or else the samples would be taken through such long intervals of time that changes in rate could not be determined at all; the objection urged against the mouth-piece of Speck also holds in respect to that of Geppert and Zuntz. This method, therefore, was rejected as not being suitable to our purpose.

¹ GEPPERT and ZUNTZ: *Archiv für die gesammte Physiologie*, 1888, xlii, p. 196; ZUNTZ and SCHUMBURG: *Physiologie des Menschen*, p. 209.

3. *Method of Hanriot and Richet.*¹ The method of these investigators is beautiful in principle. The outside air is drawn through an accurately calibrated gas-meter, is then inspired by the subject and expired through a second gas-meter. It now passes through an absorption apparatus charged with concentrated potassium hydroxide solution which dissolves the carbon dioxide, after which it passes through a third gas-meter. If v represents the volume of inspired air, v_1 that of the expired air, and v_2 that of expired air deprived of carbon dioxide, it is evident that v_1 minus v_2 represents the volume of carbon dioxide excreted, and v minus v_2 represents the volume of oxygen absorbed. This apparatus, however, offers the objection that the subject is obliged to force the air through three gas-meters thus throwing a large amount of extra work upon the lungs. Furthermore the accuracy of the method depends upon the uniform temperature of the liquid in the gas-meters and upon uniform composition of the expired air. Some experiments made by ourselves tended to show that this apparatus also was quite unsuited to our purpose, and it was therefore rejected.

The physiologist, the physicist, and the engineer have made extensive use of the graphic method. It was not until recently, however, that the chemist seriously turned his attention toward a similar application of this method to a study of the course of chemical reactions. In 1899 Ostwald,² while engaged in an investigation of the remarkable behavior of chromium toward acids, was led by the great expenditure of time required to note at frequent intervals the indications of a gas-burette, to devise an apparatus which should record automatically the rate of evolution of hydrogen. The gas was caused to flow from the generator through a long capillary tube, a pressure thus being produced approximately proportional to the rate of evolution of the gas. This pressure was caused to actuate a light lever by means of an ordinary tambour, and the curve of rate of solution of the metal was thus recorded upon a strip of paper. With this apparatus (the original chemograph) Ostwald demonstrated the periodic character of the chemical action; the effect upon the reaction of changes in temperature and of concentration of acid; the effect of numerous reagents on the periodicity of the reaction; and the synchronism of changes in the rate of chemical action with those

¹ HANRIOT and RICHEL: *Annales de chimie et de physique*, 1891, pp. 22, 495.

² OSTWALD: *Zeitschrift für physikalische Chemie*, 1900, xxx, pp. 33, 204.

of the electrical tension of the metal. A consideration of Ostwald's papers leads to the conviction that the graphic method alone could have yielded such satisfactory results in the study of a problem of this kind.

So far as the writer was aware when this research was begun, no successful attempt had ever been made to determine the rate of a chemical change by recording the movements of a balance beam.

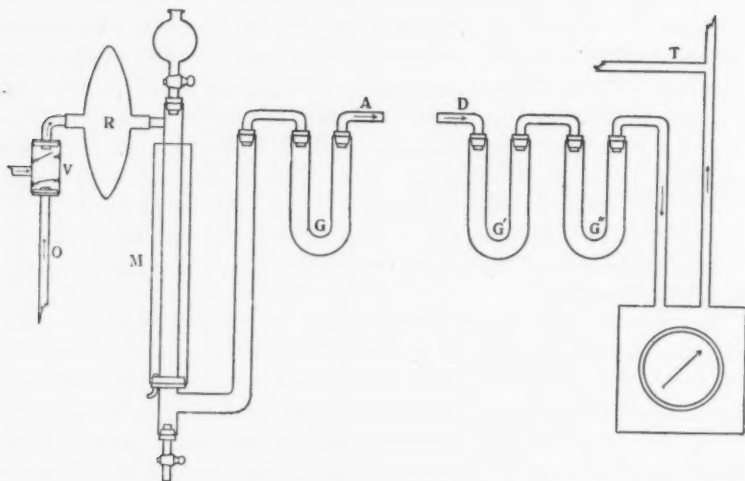


FIGURE 1. — The respiration apparatus except mask, chemograph, and pump. Out-door air enters at *O*. *R* is the bag. *M* is a condenser. The drying apparatus and guard tube are shown at *G*. *A* and *D* connect with the tubes of the chemograph. *T* is an auxiliary tube with adjustable valve, by means of which the flow of air through the main circuit may be regulated.

However, in March, 1904, Professor G. N. Stewart, of Chicago University, while examining the apparatus to be described in this paper, stated that he had some time previously demonstrated the change in weight of a dialyzer filled with cane-sugar solution and suspended from the arm of a balance in a solution of pure water. By means of a lever attached to the arm of the balance, a curve of change of weight of the dialyzer was recorded upon a drum. Professor Stewart's paper was read at a meeting of the Chemical Society of Owens College, Manchester, England, but was published only by title.

The occasion for the construction of this apparatus arose in connection with an attempt to determine the cause of the secondary rise

in the pulse rate in man during uniform muscular work. It became desirable in the progress of this research to compare the pulse curve with the curve of excretion of carbon dioxide during successive periods of rest, uniform work, and recovery. Almost at the outset it was suggested by Prof. W. P. Lombard that it might be possible by absorbing the exhaled carbon dioxide in an absorption apparatus suspended from the arm of a balance, to write a curve of carbon dioxide excreted from the lungs. Experiments were accordingly made which resulted in the foregoing apparatus.

Mask. The subject respires through a mask¹ made as follows: A copper wire about 2 millimetres in diameter is so bent as to fit over the bridge of the nose and the face inclosing nose and mouth. A piece of heavy tin is then bent in the same form, that of an ovoid about 11 centimetres in length and 8 centimetres broad at the widest part. This is soldered to the wire, making a box about 4 centimetres in depth and rather closely fitting the face. The space between this edge and the face is made air-tight in the following manner: A rubber tube, such as is used on the Townshend ether inhaler, is stretched over the wired edge of this box and by inflating the rubber and closing the tube by means of a clamp, it is possible to bring a cushion filled with air between the face and the wired edge of the mouth-piece. A sheet of rubber is stretched over the front of the box, and firmly cemented and wired in place. This is then pierced in the centre and through it passes a glass tube 1.2 centimetres in diameter, which is attached to the valve-chamber.

Especial care was taken to make the volume of the tubes between the mouth and the valves as small as possible. The mask is held firmly to the face by wide elastic bands passing around the head.

Valve-chamber. (V, Fig. 1.) The valve-chamber employed is of the same general form as that used by Zuntz and Schumburg,² except that it is made of glass instead of metal, thus permitting a view of the working of the valves. It consists of a large T tube, 20 centimetres in length and 4 centimetres in diameter, with a side tube 1.2 centimetres in diameter, to which the mouth-piece is attached. The valve seats are of cork covered with thin sheet-rubber fastened

¹ The idea of this mask was obtained from Mosso: *Der Mensch auf den Hochalpen*, p. 170.

² ZUNTZ and SCHUMBURG: *Loc. cit.*, p. 208.

on with rubber cement. The openings are about 1.5 centimetres in diameter; the valves are made of thin sheet-rubber stiffened above with a disk of very thin aluminium foil attached by means of rubber cement. The out-door air enters the lower end of the valve-chamber through a wide glass tube.

From the valve-chamber the air passes into the inner bag of a small football, holding when moderately distended about 3 litres. At each expiration this bag is somewhat inflated, but is deflated through the chemograph by the action of the pump during the next inspiration. There is thus a substantially uniform delivery of air to the absorption apparatus. At this point there is placed a *T* tube and shunt so that the air may be caused to pass directly to the pump without passing through the absorption apparatus.

Drying tubes. The apparatus for the removal of moisture consists, essentially, of a *U* tube 75 centimetres in length and 4 centimetres interior diameter, filled with coarse pumice stone saturated with concentrated sulphuric acid. This tube is followed by a guard tube *G*, about 75 centimetres long, filled in the same manner. The completeness of the action of the preceding tube may be seen in the fact that the guard tube in no case gained more than .01 gram, and usually less than 0.005 gram, during an experiment in which air saturated with water vapor and flowing at the rate of 30 litres per minute passed through the train for 30 minutes.

From the guard tube the air flows through the absorption beaker of the chemograph, passing then through two guard tubes *G'* and *G''* filled with pumice stone and sulphuric acid. The first of these tubes shows, in an ordinary work experiment, a gain of only 0.05 gram; the weight of the second remains practically unchanged. The air passes now, at the will of the operator, through a shunt tube¹ containing clear lime-water as a test for the presence of carbon dioxide, then through an Elster gas-meter and to the pump.

Suction pump. In order to relieve the lungs of the subject from the labor involved in forcing the expired air through the tubes and gas-meter, the latter is connected to the suction side of a blower, capable of drawing air through the entire apparatus at the rate of 30 litres per minute. This amount of air is sufficient for a subject engaged in moderate muscular work, but its rate is not equal to that at which air passes from the lungs during the act of expiration. The bag previously mentioned is introduced into the circuit in order to permit

¹ Not shown in the figure.

the subject to exhale freely, the air expelled at one expiration being drawn from the bag by the pump during the next inspiration.

Chemograph. The dry air containing carbon dioxide passes in the following manner into an absorption apparatus upon the balance: The tube *A* (Fig. 1) is connected by means of a short piece of very thin rubber tubing, made of a surgeon's finger-cot, with a copper tube (*B*, Fig. 2) 1.5 centimetres in diameter, which is firmly attached to the beam of the balance. The air enters directly opposite the central knife-edge, passes to the outer end of the beam, and downward opposite the lateral knife-edge through two very thin rubber connections and a glass tube (*D*, Fig. 3) into the absorption apparatus below. From the absorption apparatus the air passes upward through similar connections to the balance-tube *C*, back on the opposite side of the balance-beam to the centre, then into guard tubes, *G'* and *G''*, which have already been described. (See Fig. 1.)

Absorption apparatus. (*C*, Fig. 3.) Several forms of absorption apparatus are used in connection with the chemograph. That constructed for use in work experiments has been most employed and will be described here.

Since there was a question of removing the carbon dioxide from air flowing at the rate of 30 litres per minute during work, the absorption apparatus is necessarily large. It consists of a beaker 20 centimetres in diameter at the top, and 50 centimetres deep, with a cover of thin copper, provided with openings two centimetres in diameter, into which are fitted the inlet and outlet tubes. The air passes downward into the beaker through a thin glass tube 2 centimetres in diameter, to within about 2 centimetres of the bottom of the beaker, ending in an open space 3 centimetres deep and of a diameter equal to that of the beaker. (This open space was left because it was thought that the carbonic acid gas would thereby be more uniformly distributed throughout the whole cross-section of absorbent placed above.) The air now rises through about 5 kilograms of coarse, carefully screened soda-lime and then through glass-wool covered with phosphorus pentoxide to hold back dust and the

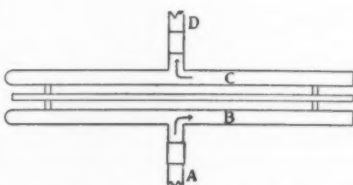


FIGURE 2.—A horizontal section through the beam and balance tubes. The air passes through those portions only which are designated by arrows.

last trace of water formed in the reaction. This beaker when charged weighs about 5.5 kilograms. It is counterpoised by another beaker of the same exterior volume filled with spent soda-lime.

Recording apparatus. In order to record the movements of the balance, there is attached to the end of the balance-beam a steel loop which engages the short arm of the light lever (*E*, Fig. 3), made of two straws placed side by side and tipped, at the short end, with a steel wire, and at the long end with a piece of parchment paper. By means of an arrangement (*F*, Fig. 3) which will be clear from the

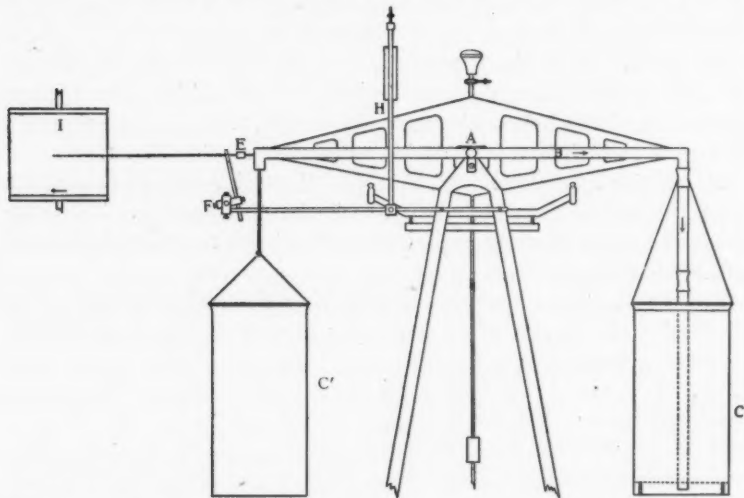


FIGURE 3.— This figure represents an elevation of the chemograph. The air enters at *A* and takes the direction shown by the arrows. *C* is the absorbing apparatus, and *C'* the counterpoise. Four gram-weights are placed upon the left beaker, and the lever-points thereby deflected upwards on the drum (*I*). The curve of carbon dioxide absorbed in *C*, is written downward to the right upon the drum.

figure, the fulcrum of the lever may be adjusted vertically, transversely, and horizontally. On the short arm of the recording lever (40 millimetres in length) there is placed a movable weight by an adjustment of which the long arm (350 millimetres in length) is made to slightly preponderate. The lever records upon the drum the movements of the balance-beam magnified nine times. Since much depends upon the accurate adjustment of the writing lever upon the paper, the kymograph is set upon a base provided with ball-bearings and with two springs working against a screw, so that the

kymograph may be rotated around a vertical axis and thus the drum be quickly and accurately adjusted to the recording lever at any time. Attached to the frame of the kymograph is a vertical brass rod¹ to which are clamped three slender brass springs which extend horizontally and whose points may be brought in light contact with the paper on the drum. The middle one marks the level of the centre of the fulcrum of the recording lever. This marker when once adjusted is, of course, never disturbed. The upper and lower ones draw lines marking the upper and lower limits of the excursion of the lever-point during calibration. They are readjusted from time to time as may be necessary.

It has been already stated that the rubber connections of the balance were made very light in order to avoid, as far as possible, interference with the free movements of the balance. In order now to be able to write a curve of considerable length, representing, for example, a mass of five grams, it became necessary to diminish by some means the sensibility of the balance while interfering as little as possible with the uniformity of its movement. There was, therefore, attached to the frame of the balance, about ten centimetres from the central knife-edge, a steel yoke (*H*, Fig. 3) passing over the beam. From this yoke there was suspended a coil, five centimetres in length and about one centimetre in diameter, made of phosphor-bronze wire approximately half a millimetre in diameter. This coil was attached at its lower end to the upper side of the beam of the balance by means of a hook, which together with the yoke could be set at any desired distance from the central knife-edge. A set screw, with check-nut by which the upper end of the coil is attached to the yoke, admits of an adjustment of its tension at the will of the operator.

Adjustment of tension. The balance is brought into equilibrium with spring disconnected. The spring is now attached to the beam and brought into a state of tension by turning the screw at the upper end of the coil. Weights are now placed upon the pan on the same side until equilibrium is again restored. In most of the work done with this apparatus the spring has had an initial tension of four grams.

We have here a combination of the beam and the torsion balance. This apparatus was subjected to a series of careful tests in order to

¹ Not shown in the figure.

ascertain whether the records inscribed by it upon the blackened paper were of any value.

Tests of the balance.¹— I. *Test with all connecting tubes removed and with a load of 5.5 kilos.* In order to ascertain the accuracy with which weighings could be made on this balance, various objects were weighed upon the balance, and then to tenths of a milli-

TABLE I (A).

	Series I.	Series II.	Series III.	Average.
Upper gram	15.4	15.1	14.7	15.06
Second gram	15.3	15.0	15.0	15.1
Third gram	15.2	15.3	14.7	15.1
Fourth gram	14.6	14.6	14.7	14.63
Fifth gram	(14.2)	(13.9)	(13.3)	
Total deflection	60.5	60.0	59.1	

TABLE I (B).

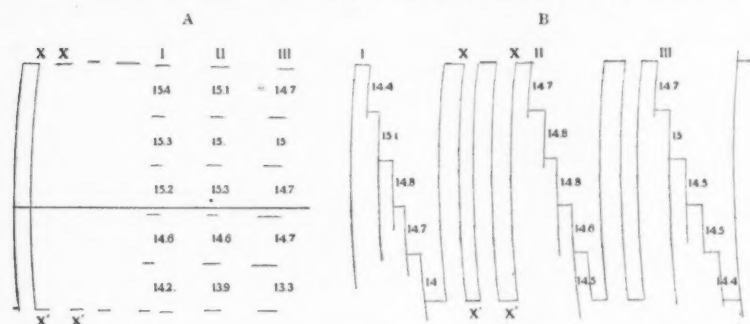
	Series I.	Series II.	Series III.	Average.
Upper gram	14.4	14.7	14.7	14.6
Second gram	15.1	14.8	15.0	15.03
Third gram	14.8	14.8	14.5	14.7
Fourth gram	14.7	14.6	14.5	14.6
Fifth gram	(14.0)	(14.5)	(14.4)	
Total deflection	59.0	58.9	58.7	

gram upon a fine balance. It was found that the variation of the two weights was, in no case, more than 0.0103 gram and averaged 0.005 gram.

2. *Calibration of the apparatus.* The tubes and recording lever were now adjusted, and a careful test was made of the amount of vertical deflection of the end of the recording lever produced on the

¹ This balance was made by RÜPRECHT, and is a most satisfactory instrument.

blackened drum by a mass of five grams. This was done as follows: The balance was brought into equilibrium. The beam was now arrested and five gram-weights were placed upon the counterpoise beaker; this produced an angular deflection of the beam of about $1^{\circ} 45'$, and a vertical deflection of the recording lever-point of about 73 millimetres. After a delay of about 30 seconds to allow the lever to assume its position of rest, the screw controlling the position of the drum was carefully turned until the blackened paper was brought into light contact with the writing lever, and the kymograph was started and allowed to run until a short horizontal line had been drawn by the point of the recording lever upon the paper. The beam was now arrested, the weights removed, the beam again released,



FIGURES 4 A, and 4 B. — These figures show the results of calibration of the chemograph with 5 gram-weights. The distances XX' are the vertical deflections of the recording lever-point for a mass of 5 grams added to the pan. The numbers represent the vertical deflection in millimetres of the lever point for one gram. A calibration of at least one series is made at the beginning of each experiment, and often at the close also.

and the writing lever again allowed to come into a position of equilibrium. The kymograph was now started as before and a second light horizontal line drawn upon the paper. The vertical deflection of the writing point is a measure of the mass of five grams. This process was repeated many times, the weight being alternately added and removed to find out the accuracy with which the point of the writing lever returned to the same level on the drum. It was found that at the beginning of work, after the apparatus had stood for some hours, there was some irregularity at the first two or three movements of the beam. However, after a few minutes the movements became quite uniform. Starting, now, from the highest position of

the recording lever, the gram weights were removed, one by one, the position of rest of the point of the lever being marked at each step by a short horizontal line as in the preceding case.

Fig. 4 A and Table I (A) show the results of one of these calibrations in which are given the deflections due to one gram. Omitting the lower or fifth gram of each series in I (A) and we have the following averages: 15.06-, 15.1-, 15.1-, 14.6. The total deflection for four grams is, in the three series, 60.5, 60, and 59.1 millimetres, respectively. The extreme variation in deflection for four grams is 1.4 millimetres or 2.3 per cent, and the greatest variation from the average is 0.7 millimetre or 1.15 per cent. The greatest variation in deflection for a single gram (omitting the lower or fifth gram in each series) is 0.8 millimetre or 5.2 per cent; the greatest variation from the average is 0.36 millimetre or 2.4 per cent. Figure 4 B and Table I (B) show the results of a calibration of the same apparatus, with slightly different tension in the spring. In this case the greatest variation in deflection for 4 grams and 1 gram are 0.3 millimetre or 0.5 per cent, and 0.6 millimetre or 4 per cent, respectively, and the greatest variation from the average, 0.2 millimetre or 0.32 per cent, and 0.38 millimetre or 2.5 per cent. The results of numerous calibrations showing that the deflection for the lower or fifth gram invariably gives low values, the use of this portion of the arc has been discontinued.

3. *The next test was carried out as follows:* A small crystallizing dish, previously weighed upon a fine balance, was placed upon the right beaker and the balance brought into equilibrium. Four gram-weights were now added to the left beaker and the point of the lever thereby deflected vertically about 58 millimetres upon the drum. The usual calibration with 4 grams having now been made, the drum was started and a slow stream of mercury was allowed to flow into the crystallizing dish from a simple apparatus with capillary delivery-tube. There was thus described upon the drum a short horizontal line, followed by a more or less regular curve inclining downward toward the right. Finally, when about two grams of mercury had been allowed to flow in this manner into the crystallizing dish, the addition of mercury was discontinued, and the curve caused to end in a horizontal line. The beam was now arrested and the crystallizing dish removed. The vertical deflections of the writing lever due to the successive addition and removal of four grams in the initial calibration process were now determined. The

average of these values is, of course, the graphical equivalent of four grams. From this there was readily obtained the modulus of the balance, viz. the number of milligrams represented by one millimetre of vertical distance upon the drum. The vertical distance between the initial and the final positions of the writing lever in the experiment with mercury was now measured, and

TABLE II.
SHOWING THE RESULTS OF CALIBRATION OF BALANCE WITH MERCURY.

Vertical deflection for one gram.	Modulus ¹ M.	Initial height writing point = h.	Final height writing point = h'.	Weight graphically determined (h-h') M.	Weight on fine balance.	Error.	Per cent error.
millimetres		millimetres	millimetres				
11.63	0.0859	82.5	55.9	2.287	2.273	+0.014	+0.61
11.63	0.0859	55.9	26.4	2.537	2.5695	+0.0326	+1.26
11.63	0.0859	83.5	58.5	2.148	2.1624	-0.0144	-0.66
11.63	0.0859	58.5	34.6	2.055	2.0707	+0.0152	+0.70
14.57	0.0686	88.0	66.9	1.447	1.4331	+0.014	+0.98
14.57	0.0686	66.9	34.5	2.2237	2.2172	+0.0065	+0.29
14.57	0.0686	87.8	59.9	1.9149	1.9092	+0.0057	+0.3
14.57	0.0686	59.9	24.3	2.4434	2.4424	+0.001	+0.04
14.58	0.0686	88.0	76.0	0.823	0.847	-0.024	-2.8
14.59	0.0686	76.0	62.3	0.9396	0.9282	+0.0114	+1.23
14.59	0.0686	73.3	59.3	0.953	0.9488	+0.0032	+0.40
14.59	0.0686	59.3	42.7	1.139	1.177	+0.0213	+1.9

¹ Experiments 1-4 were made by the use of a brass spring; the following experiments with a phosphor-bronze spring of quite a different tension. This accounts for the widely different values of the modulus.

the weight of mercury added, obtained by multiplying the modulus by this value. Finally the crystallizing dish with its contents was reweighed upon a fine balance and the weight of the mercury thus determined compared with that obtained by the graphical method.

The results of a series of such tests are shown in Table II, in which there are given: Vertical deflection in millimetres of lever-point for

one gram added to the pan; Modulus M or the weight in grams corresponding to one millimetre deflection; Initial height of writing lever-point " h "; Final height of lever-point " h' "; Weight of mercury as graphically determined, $(h-h')$ M ; Weight of mercury as determined on fine balance; Error $+$ or $-$; and per cent of error. It will be observed that the error was generally positive and varied in the different experiments between 0.001 and 0.0326 gram, the average error being about 0.014 gram or approximately 0.7 per cent.

4. *Test with a uniform current of carbon dioxide.* A current of carbon dioxide, as uniform as possible, was now caused to flow for seven minutes through the apparatus, the drum meanwhile revolving uniformly. The result was a smooth curve about 30 centimetres in length, which was a very close approximation to a straight line, measurements showing that at no point was the deviation from straight line greater than 0.7 millimetre. This experiment was repeatedly performed with practically the same result.

It is evident that when the recording lever has reached the lower limit of the arc the beam may be arrested, and additional weights added without interrupting the experiment and with a loss of only a small portion of the curve. This may be repeated for hours, thus enabling the operator to determine both the *course of the reaction* throughout its whole extent and the total weight of gas absorbed.

The method of determining the rate of the reaction during any period is as follows: With a radius equal to the length of the long arm of the recording lever, and with the proper points on the central reference line as centres, arcs are drawn cutting the time line at the beginning and the end of the desired period, and also the curve of carbon dioxide. The vertical distance between the two intersections of the carbon dioxide curve by these arcs is the measure of the amount of that gas absorbed during the time cut off below. The modulus of the apparatus having been determined as described earlier, the rate of absorption between the desired limits is readily determined.

5. *Test with a weighed quantity of carbon dioxide.* A series of experiments was now carried out with carbonic acid gas. There was set up a carbon dioxide apparatus consisting of a small fractionating flask provided with a dropping funnel and delivery tube, to which was attached a drying tube filled with pumice stone and sulphuric acid. Into this flask there was brought a quantity of a saturated solution of sodium carbonate, while sulphuric acid was placed in the dropping

funnel. The apparatus was now carefully weighed, after which it was attached to the drying tube of the chemograph, a current of pure air free from carbon dioxide was drawn through it at the rate of half a litre per minute, and the sulphuric acid was slowly dropped upon the carbonate. The gas thus produced, diluted with seven litres per minute of purified outer air was drawn through the absorption apparatus, the kymograph drum meantime revolving at a uniform rate. This experiment was repeatedly tried, with the result that the maximum error was 2.8 per cent.

It is evident that this form of chemograph may be used in a study of the course of many chemical reactions in which gas or vapor is evolved, since the apparatus will write a curve of *loss in weight* as readily as of *gain in weight*. It is only necessary to place the generator upon the pan of the balance, to make the usual adjustments, and allow the process to continue as long as desired. A tracing of the course of a reaction in which there is an escape of hydrogen will, perhaps, not be practicable with this apparatus, on account of the relative lightness of that gas. However, the curves of rate of loss of water, ammonia, carbon dioxide, etc., will be readily written. Some work in which the apparatus has been used in this manner has already been done, the results of which will be published later.

THE EXCRETION OF CARBON DIOXIDE RESULTING FROM BICYCLING.

1. *General form of the curve.*—About twenty experiments were made to find the general course of the changes in output of carbon dioxide resulting from work. The work was done upon the stationary bicycle described in the papers already published in this series.¹ It is sufficient to say here that the machine is driven by the subject at a chosen rate which is recorded continuously, and that the resistance of the machine can be adjusted at any desired amount. The graphic record of carbon dioxide excretion is taken on a slowly revolving drum along with records of respiratory movements, revolutions of the bicycle, and time in seconds. On a loop of paper passing around two drums and moving more rapidly, the pulse is recorded along with a time curve, giving seconds. In beginning an experiment, the subject puts on the breathing mask, mounts the wheel, the pneumograph and pulse tambour are put on and tested, and then the mask is con-

¹ BOWEN: *Loc. cit.*, p. 465.

nected to the tubes leading to the apparatus for determining carbon dioxide. The drums are started, and the subject sits quietly on the bicycle until sufficient records have been made to show the normal rate of pulse, breathing, and output of carbon dioxide. Then at a signal from the experimenter he begins to drive the wheel in unison with a metronome placed before him, and the records continue. The balance is handled as described in the preceding section. The electric signal connected with the bicycle indicates the moment of starting and stopping, and the speed of revolution. On cessation of the work the records continue until the carbon dioxide has resumed approximately the rate before the work began, as indicated by the slant of the line described by the recording lever. The experiment then ends. A record taken in this way is shown in Fig. 5.

By looking at Fig. 5 one can observe in a general way the effect of the work on the output of carbon dioxide. Beginning at the left, the

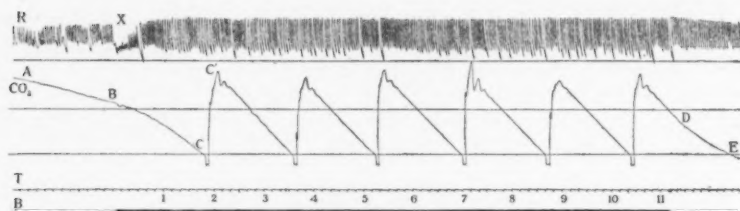


FIGURE 5. — Graphic record of carbon dioxide excretion during bicycling. Read from left to right. *R*, respiratory movements. *CO₂*, record made by chemograph. The descending lines, as *AC*, are due to accumulation of carbon dioxide in absorbing beaker. The ascending lines, as *CC*, are due to addition of weights by operator. *T* is the time, marked every ten seconds. *B*, revolutions of the bicycle crank.

nearly straight slanting line *AB*, drawn by the lever connected with the balance, indicates the rate of output during rest. Then, when work begins, the increased slant of *BC* indicates an increased excretion, and this continues to increase for about two minutes. From this time to the end of the working period the slant of the line remains about the same, indicating uniform output of carbon dioxide. As soon as work stops there is an immediate change in the slant *DE*, showing the diminished excretion. There are some irregularities in the line we are considering, but they are not sufficient to interfere with an accurate reading of the curve. These disturbing features result from vibrations of the balance caused by placing on of weights, and by vibrations of the building due to various causes.

A more accurate idea of the changes in question can be obtained from Fig. 6, which was obtained from the record of Fig. 5 by careful measurements and plotting. This figure also contains the plotted curve of pulse rate, which was recorded simultaneously on another paper. The pulse curve shows plainly a rapid primary rise, *ab*, a plateau, *bc*, and a slow secondary rise, *cd*. The curve of carbon dioxide rises rather rapidly during the first two minutes, which includes the period of rapid rise of pulse rate and a part of the plateau. During the remainder of the working period the output is seen to be practically constant, although the pulse rate is rising for the latter half of the time. On cessation of work the output diminishes until at the end of two minutes it has returned to practically the original rate. At the same time the pulse rate, although falling rapidly at first (*de*) is oscillating about a rate (*ef*) 20 per cent above the normal. These results are fair examples of those obtained in the series. A few experiments covered working periods of from 30 to 45 minutes. Here the carbon dioxide was determined during the critical stages and during only one minute of each five through the most of the period.

2. *Latent period.*—A large number of experiments were made to determine how soon after work begins the increase in production of carbon dioxide begins to show itself in the expired air. Fig. 7 shows a group of records taken in the course of these experiments. The procedure is as follows: the subject, sitting quietly on the bicycle, breathes into the apparatus for about 30 seconds, the output of carbon dioxide being recorded on the drum, giving the line *mn* in curve *A*. He then begins driving the bicycle, the time of starting being accurately indicated by the marker *B*. The experiment continues only long enough to show a definite increase in the output of carbon dioxide. After fixing the record in shellac, the point *n* on the curve of carbon dioxide, where the line first changes its direction as the result of the work, is revolved to the base line with a radius equal to the length of the long arm of the writing lever, so as to avoid error

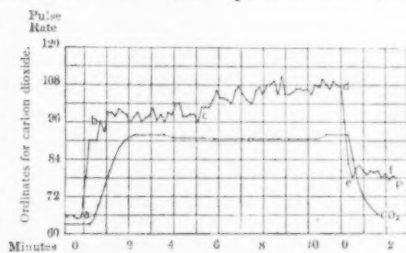


FIGURE 6.—Plotted curves of pulse rate and excretion of carbon dioxide from same experiment as Fig. 5. Broken line, pulse rate; solid line, carbon dioxide. Ordinates give pulse-beats per minute and grams of carbon dioxide per minute.

due to rotation of the lever on its axis. Now the number of seconds intervening between the beginning of work and the resulting change in output of carbon dioxide can be readily obtained from the time record, *T*.

To find the result desired we must deduct from the time found in the manner just described the time occupied by the passage of the exhaled air from the mouth and nostrils through the mask and connecting tubes, and sufficient additional time to collect in the soda-lime enough carbon dioxide to overcome the inertia of the balance. The time to be deducted is found as follows: while sitting quietly upon the bicycle, with the record in progress, the subject holds his breath for several seconds. The result is shown in curve *C* of Fig. 7. The pneumograph curve at the top of the record shows when the balance is held. Soon the lever which records the movements of the balance ceases to fall, as indicated by its writing a level line *u v*. When the subject begins to breathe again the pneumograph curve shows the exact moment of the first expiration, and the time from this point to the point *v*, where the carbon dioxide lever first begins to fall again is the time of delay due to the apparatus. From a large number of tests this time was found to be close to 6 seconds. This delay evidently depends upon the rate at which air is drawn through the apparatus by the suction pump. For this reason all the experiments on latent period were made with the air moving at the uniform rate of 20 litres per minute.

Making the deduction of 6 seconds in the case recorded in curve *A* of Fig. 7, we obtain 5 seconds as the latent period. This period varied considerably in different cases, all the way from 3 to 14 seconds. Now it is evident that none of these results is long enough to correspond with what we hoped to find, viz., the time required for the carbon dioxide formed in the muscles at the time of the first muscular contraction to reach the outside air. The carbon dioxide must first diffuse into the blood from the tissues where it is formed, then traverse the venous half of the systemic circulation, the right side of the heart, and the arterial half of the pulmonary circulation, and finally diffuse into the air of the alveoli before any of it can appear in the breath. From the latest conclusions of Stewart¹ and others who are considered as authorities on the time of the circulation, we learn that from 15 to 20 seconds is the least possible time for the blood to travel

¹ STEWART: Manual of physiology, Philadelphia, 1900, p. 124.

this distance, to say nothing of the diffusion time. We must evidently account for the shortness of the latent period thus found.

The explanation is provided by two facts which appeared in the course of these experiments, showing that the prompt increase on beginning work is due to an increase in the depth of respiration which accompanies the beginning of work. The fact that there is a prompt increase in depth of respiration when work is begun has been mentioned by Tigerstedt.¹ We discovered the increase in this way: in the description of apparatus in the preceding section, mention was made of a light rubber bag inserted into the air circuit between the mask and the absorbing beaker for the purpose of holding temporarily a part of the air expelled at each breath, and thus preventing excessive pressure in the lungs during expiration. With each breath exhaled the puff of air partly inflates this bag, and the

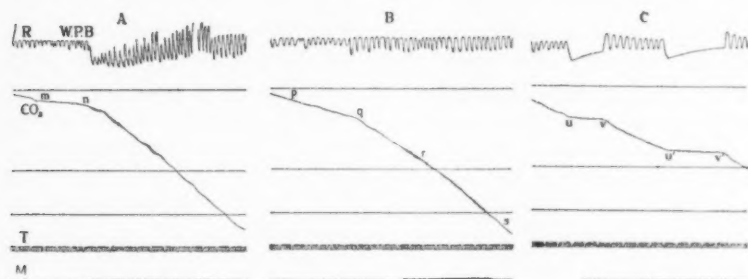


FIGURE 7.—Excretion of carbon dioxide during work and during modified respiratory movements. *R*, respiratory movements; *CO₂*, record of chemograph; *T*, time in seconds; *M*, bicycle. Curve *A* shows changes due to beginning work; curve *B*, deepened respiration followed by work; curve *C*, holding the breath.

action of the suction pump empties it again while the next breath is being inhaled. Now, by a careful observation of the amount of inflation of the bag, one can detect any considerable change in the volume of successive breaths. Upon watching the bag at the time of beginning work it was noticed that a marked increase in the volume of each breath occurs promptly in almost all cases as soon as the subject begins work.

Upon observation of this fact the question at once suggested itself, how much effect a voluntary increase in depth of respiration would have if not associated with work at all. The answer is given by

¹ TIGERSTEDT: *Loc cit.*, p. 170.

curve *B* of Fig. 7. Line *p q* indicates the rate of output of carbon dioxide with normal breathing. At *q* the subject began deep inspirations, with the increased output indicated by the greater slant of the line *q r*. This marked increase, which is temporary, is to be explained in all probability by the greater amount of alveolar air, richer in carbon dioxide, which is exhaled during deeper respiration. The fact thus noted suggested in turn a method of attacking the original problem. If a subject ordinarily increases the depth of his respiration on beginning a piece of muscular work, and thus introduces an element that obscures the point at issue, viz., how soon the direct effects of the work show themselves, why not voluntarily increase the breathing before beginning the work, and thus separate the two effects? Several experiments were made in this way and the results are quite satisfactory. Curve *B* of Fig. 7 shows the result in one instance. The effect of the increased respiration is clearly marked, the new rate of output *q r* being sharply defined from the normal rate *p q* preceding it. The further increase on beginning work (*r s*) is not so prompt in its appearance and comes on more gradually, reaching its maximum after a minute or more, depending on the work. In these experiments the latent period of increase on starting the work was from seventeen to twenty-two seconds. It is evident that the latent period will vary with the rapidity of the circulation and also with the rapidity of diffusion, so that a more definite figure is not to be expected.

DISCUSSION OF RESULTS.

These results agree in the main with those obtained by others, as far as they lie in the same field. Johansson¹ found a uniform output of carbon dioxide during uniform muscular work, but his determinations were made for periods of several minutes' length, while our results show the output from minute to minute. Our work involved more extensive muscle area than his, but on the other hand we did not investigate the question during breathlessness and extreme fatigue, because the apparatus that we used did not supply air enough for a subject doing excessive work. We see no reason, however, why with a larger apparatus the same method would not apply in all such cases.

In studying the manner in which the output of carbon dioxide

¹ JOHANSSON: *Loc. cit.*

changes at the beginning and end of the working period, we believe we have entered a new field. Zuntz and Schumburg,¹ Tigerstedt,² Johansson, and Katzenstein³ state definitely that they did not begin estimating the carbon dioxide for some time after the work was in progress, and no investigator, so far as we have noticed, has studied this stage before. When we consider the manner in which the change in formation of carbon dioxide occurs in the muscles, and the manner of its elimination from the body, our results seem entirely consistent with the conditions. Since the work begins suddenly at full speed and force, the formation of carbon dioxide in the muscles must jump instantly from the normal rate to the maximum rate during the work. Since the gas must first diffuse into the blood and then be carried to the lungs before elimination can take place, there should be a latent period of a little more than half the time required for a complete circuit of the blood before the first waste product formed during work can be exhaled. The latent period of 17-22 seconds found here is in full accord with the work of Stewart previously mentioned. Since the latent period is dependent upon the rapidity of the circulation, which is different at different times, even in the same subject, a variation of several seconds is to be expected.

The sudden increase in carbon dioxide in the tissues produces a gradual increase in output from the lungs, because of the manner of elimination. The diffusion process tends to round off the sharpness of the change, and the transportation in the blood stream does so still more, since the portion of the gas that is absorbed in the blood that traverses the centre of the stream will reach the lungs considerably sooner than that following the walls of the vessels; there are also routes of different lengths from different muscles. The maximum output will evidently be reached when the blood leaving the muscles after work has begun and travelling by the slowest and longest route, finally gives off its gases in the lungs. Upon cessation of work the production of carbon dioxide drops back to the normal rate as suddenly as it arose, but the reasons just given show why there is again a latent period, and then a gradually diminished output. A temporary increase in output sometimes found at the instant work ceases is readily accounted for by the fact that the respirations, which are more or less hindered by the muscular con-

¹ ZUNTZ and SCHUMBURG: *Loc. cit.*, p. 212.

² TIGERSTEDT: *Skandinavisches Archiv für Physiologie*, 1895, vi, p. 170.

³ KATZENSTEIN: *Archiv für die gesammte Physiologie*, 1891, xlix, p. 338.

tractions involved in driving the wheel, become somewhat deeper when this hindrance ceases, since the tissues are still as fully saturated with carbon dioxide as at any time, so that there is for a few moments no diminution of the respiratory need.

Fig. 6 shows a very slight diminution of the output of carbon dioxide during the latter part of the working period. This is probably accidental, as it does not occur in all cases, some experiments showing a slight increase. The change may be due to a change in the manner of doing the work — the subject driving the wheel with slightly less muscular expenditure at some times than at others. The great variation in internal work that may occur in the doing of a certain amount of external work has been pointed out in another paper of this series.¹ One possibility thought of in connection with the carbon dioxide curve in Fig. 6 is the warming up of the absorbing beaker by continuous respiration through it, thus causing the air within it to become lighter, tending to counteract the increased weight due to carbon dioxide. As the experiments made gave a slight increase during work as frequently as a decrease, this was not considered likely. A group of longer experiments in which the air was drawn through the absorbing beaker only one minute of each five showed no difference in this respect, also indicating that change of temperature of the absorbing beaker was not the cause of the irregularity. The change, however, is so slight as to have no effect on the general interpretation of the results, whatever may be the cause.

When we compare the curves of pulse rate and carbon dioxide, we see plainly that the primary rise of pulse frequency coincides in time approximately with the rise in output of carbon dioxide, and the same can be said of the corresponding fall. The short latent period of the pulse shows, as has been stated elsewhere,² that if the production of carbon dioxide is in any way responsible, even in part, for the change in pulse rate when work begins, the influence must be brought to bear through nervous channels, rather than by an effect of the gas itself upon the heart or the cardiac centres. We see no reason why the prompt increase in heart action may not be due in part to sensory impulses arising in the muscles as a result of the waste products suddenly set free there, as advocated by Athanasiu.³

¹ BOWEN: *Loc. cit.*, p. 485.

² BOWEN: *Loc. cit.*, p. 475.

³ ATHANASIU and CARVALLO: *Archives de physiologie*, 1898, pp. 554, 567.

The results obtained show no evidence of any relation of cause and effect between the production of carbon dioxide, and the secondary rise in pulse rate. In Fig. 6 we see that the output of carbon dioxide is constant during the entire period of the secondary rise of pulse rate, while the secondary fall of pulse rate during recovery is continued for a long time after the output of carbon dioxide has returned to the normal. The lack of correspondence in the two curves practically amounts to a demonstration that the secondary changes in pulse rate have nothing to do with the production of carbon dioxide and its elimination from the system.

SUMMARY.

1. The problem of finding the changes in rate of output of carbon dioxide resulting from muscular work and other causes is practically solved by the method used in this research.
2. The latent period of increase in output of carbon dioxide from the lungs in case of beginning work is in the close vicinity of twenty seconds, and the increase reaches its maximum in about two minutes.
3. The output of carbon dioxide from the lungs is practically uniform from minute to minute during uniform muscular work, after the blood has had time to take part fully in the process of elimination.
4. Upon cessation of work the output of carbon dioxide decreases to the normal amount in about the time occupied by its increase, and after a like latent period.
5. The results obtained show no indication of any connection of cause and effect between the production and elimination of carbon dioxide and the secondary rise of pulse rate.

The writers desire to acknowledge their great indebtedness to Prof. W. P. Lombard, without whose assistance this research could not have been carried on.

ON THE ABSORPTION AND UTILIZATION OF
PROTEIDS WITHOUT INTERVENTION OF THE
ALIMENTARY DIGESTIVE PROCESSES.

BY LAFAYETTE B. MENDEL AND ELBERT W. ROCKWOOD.

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THE experiments which have given rise to this paper were originally planned to ascertain the fate of proteids introduced into the circulation without the intervention of the alimentary digestive processes. For a variety of reasons proteids of vegetable origin were selected for study. They are naturally foreign to the blood; their relatively easy preparation and purification offers advantages over the more commonly employed products from animal sources; and, furthermore, the recent investigations of the vegetable proteids selected indicate that they are by no means identical in chemical make-up with the serum- or tissue-proteids. Nevertheless, the vegetable proteids are common constituents of the food of animals, and are unquestionably utilized by these organisms. The problem at once arises, whether—and if so, to what extent—these foreign proteids must be subjected to digestive changes before they can be assimilated.

The teaching regarding the transformations which proteids undergo in the alimentary tract preliminary to absorption has lately experienced considerable modification. The much-quoted experiments of Voit and Bauer, Eichhorst, Czerny and Latschenberger, and others¹ were interpreted to indicate a direct absorption of proteid from the intestine without previous digestive changes. Clinical experience with nutrient clysters contributed to strengthen this view. Accordingly various writers have assumed that while peptonization may facilitate the rate of absorption, the native proteids can readily be used without the intervention of proteolytic enzymes, provided they are introduced in soluble form. Indeed, Bunge has insisted that there is no *a priori* ground for supposing that proteid is not absorbed

¹ A review of the literature on this subject by I. MUNK will be found in the *Ergebnisse der Physiologie*, 1902, i, (1), p. 310.

unchanged. If, he says, fat droplets visible under the microscope, and even entire leucocytes, can leave the blood-capillaries and travel through the tissues, why may not a proteid molecule find its way through the capillary wall?

The more recent literature on this subject presents a radical change in the views expressed. The discovery of the crystalline cleavage products of the proteids in the intestinal contents of the living animal, and especially Cohnheim's observations¹ on the existence of the peculiar enzyme *erepsin*, capable of breaking down the more soluble proteid derivatives (proteoses and peptones) into crystalline products which no longer respond to typical proteid (biuret) tests, have brought new ideas. We are now told that the organism must synthesize the proteids peculiar to its tissues and fluids; that assimilation finds expression in the reconstruction of amido-acids, diamido-acids, etc., into new molecules. In support of this the experiments of Loewi² and of Henderson and Dean,³ indicating the maintenance of nitrogenous equilibrium upon a diet free from proteids, but containing the products of profound proteolysis, are advanced.

It must be admitted that there is something attractive in the theory which assumes a complete breakdown of the food-stuffs prior to their anabolism into living tissue or circulating blood constituents. For it is less difficult to conceive how the organism can construct from these fragments the tissues peculiar to itself, and maintain its chemical integrity, although the ingesta may vary widely in composition. The available experimental data are, however, by no means adequate to afford a definite answer to the problems presented. The non-proteid nitrogenous cleavage products are not found in the blood stream;⁴ the presence of proteoses in the blood under normal conditions has not been successfully demonstrated;⁵ and the toxic

¹ COHNHEIM: *Zeitschrift für physiologische Chemie*, 1901, xxxi, p. 451; 1902, xxxv, p. 134; 1902, xxxvi, p. 13.

² LOEWI: *Archiv für experimentelle Pathologie und Pharmakologie*, 1902, xlviii, p. 303.

³ HENDERSON and DEAN: *This journal*, 1903, ix, p. 386. Since the above was written, somewhat different conclusions have been arrived at by ABDERHALDEN and RONA: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 528.

⁴ Cf. KUTSCHER and SEEMANN: *Zeitschrift für physiologische Chemie*, 1902, xxxiv, p. 528.

⁵ The experiments of ABDERHALDEN and OPPENHEIMER: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 155, seem decisive on this point, in opposition to the positive results recorded by EMBDEN and KNOOP: *Beiträge zur chemischen Physiologie*, 1902, iii, p. 120, and LANGSTEIN: *Ibid.*, 1903, iii, p. 373.

properties of the latter when introduced directly into the circulation have been clearly established in this laboratory by Underhill,¹ despite the contrary views which Pick and Spiro² have advanced. Furthermore, the unaltered food proteids are ordinarily not present in the general circulation in quantities detectable by chemical means; and observations like those of Abderhalden, Bergell, and Dörpinghaus³ suggest that even under the unusual conditions of protracted hunger the blood- and tissue-proteids preserve their chemical individuality.

THE ABSORPTION OF PROTEIDS FROM THE SMALL INTESTINE.

To the list of publications on the absorption of native proteids, as reviewed in the cited monograph by Munk on absorption, may be added those of E. W. Reid.⁴ In dogs, he noted a disappearance of the animals' own serum from loops of the gut. All of the previous investigators have studied the behavior of either egg-albumin, serum proteids or other proteids obtained from animal sources. We have made use of the crystallized vegetable proteid edestin (obtained from hemp-seed)⁵ and of casein, as distinctive types of foreign native proteids. For comparison, simultaneous trials were also made in some cases with edestin-proteoses and caseoses.

Methods employed.—Unfed dogs were used in these experiments. Morphine sulphate was given hypodermically in the dose of about one centigram per kilo of body-weight, the operative procedures being carried out during ether anæsthesia. A loop of small intestine was withdrawn from the abdomen, carefully packed in cotton kept moistened with warm physiological saline solution, and thoroughly irrigated with the latter until the wash water ran perfectly clear from the lumen, the intestine being very gently massaged to facilitate the emptying of it. Ligatures were placed at two suitable places, so as to interfere as little as possible with the mesenteric circulation. The intestine was then tied at the lower ligature, the warm proteid solution introduced slowly from a burette through a small

¹ UNDERHILL: This journal, 1903, ix, p. 345.

² PICK and SPIRO: *Zeitschrift für physiologische Chemie*, 1900-1901, xxxi, p. 237.

³ ABDERHALDEN, BERGELL, and DÖRPINGHAUS: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 153.

⁴ REID: *Journal of physiology*, 1895, xix, p. 240; *Proceedings of the Royal Society, London*, 1899, lxxv, p. 94.

⁵ By OSBORNE's method. See CHITTENDEN and MENDEL: *Journal of physiology*, 1894, xvii, p. 50.

incision, and the second ligature tied to retain the contents of the loop. As soon as the intestine had been ligated at both sides of the incision, the loop was replaced in the abdomen and the wound closed. After a suitable interval, the animals were bled to death, the loop of intestine excised and measured, and the adherent blood wiped away. The contents were then carefully withdrawn. The fluid had nearly always disappeared and it became necessary to remove the residual precipitate of proteid mechanically (by gentle scraping and washing). The contents were dissolved in salt solution or hot water, and nitrogen estimated quantitatively in an aliquot portion by the Kjeldahl method. That this procedure involved no serious error through introducing contaminations from the intestinal wall was shown in experiments in which *the unabsorbed edestin was recrystallized and weighed as such*. In some experiments control (empty) loops, as well as loops containing proteoses, were simultaneously ligated in the same animal.

The *edestin solutions* used were quite concentrated, sufficient sodium carbonate being added to prevent precipitation at the temperature of the body. The exact content of proteid was determined from N-estimations by Kjeldahl's method. The edestin was not materially altered by the alkali used, since it could be recovered from the gut in typical crystalline form, and was identified in this way. The *edestin-proteose* solutions contained mixtures of proteoses, peptones, etc., prepared as follows: Recrystallized edestin was digested with pepsin-hydrochloric acid until no precipitate formed on neutralization. The concentrated solutions were precipitated with alcohol. The aqueous non-coagulable solution of this precipitate, containing 1.28 gm. N per 100 c.c., was used. *Acid edestin* was prepared by allowing 0.4 per cent hydrochloric acid to act upon crystallized edestin for several days. It was precipitated by neutralization and redissolved in warm 0.9 per cent sodium carbonate solution. *Casein* (caseinogen) was prepared by Hammarsten's method, being re-precipitated twice, and was added in excess to a dilute sodium carbonate solution. The filtered and very slightly alkaline casein solution was used in the trials. A solution of *caseoses* was prepared by the same methods as were applied to the edestin products.

Admitting that the absorption figures obtained by these methods tend to be too low rather than high, owing to the technical difficulties in recovering the unabsorbed proteids, our protocols indicate that both edestin and casein disappeared only slowly from the intestine within a period of four to five hours. This is in marked contrast with the findings in the case of the proteoses from the same sources, studied in the same animals. It seems unnecessary to repeat all the

details of the experiments here. A few typical protocols will indicate the mode of procedure; the more important observations are briefly summarized in a table below.

Typical protocols. — *Experiment 7. Relative absorption of edestin and edestin-proteoses.* Dog of 7.2 kilos. Loops of intestine (as indicated in the summary below) were prepared in the upper and lower parts of the small intestine, with an empty control loop of 38 cms. between them. Fifty-eight c.c. of edestin solution containing 0.587 gm. N or 3.12 gms. edestin were introduced into the upper loop (61 cms. long). The lower loop of 51 cms. received 23 c.c. of edestin-proteose solution. The animal was killed by bleeding, after 5½ hours. The intestinal mucosa presented a normal appearance. None of the ligated portions contained any liquid contents. From the edestin loop hard, curdy masses were removed by gentle scraping and washing, and treated with warm salt solution in which most of the material dissolved.¹ By diluting the filtered solution with warm water and cooling gradually, typical edestin crystals were obtained. A nitrogen estimation on a portion of the solution indicated a recovery of 0.35 gm. N, or 62 per cent; by weighing the crystals recovered from an aliquot portion, an edestin content of 69 per cent was calculated. The proteose loop was washed out with water, slightly acidified with acetic acid, and heated to precipitate any coagulable proteid. The filtrate, which gave a slight biuret reaction, contained 0.052 gm. N of the 0.295 gm. introduced, or 17 per cent.

RECOVERED.

Edestin	69 per cent.
Edestin-proteoses	17 "

Experiment 12. Relative absorption of sodium casein and caseoses. Dog of 11.5 kilos. Sodium casein and caseose solutions were introduced into two separated loops as indicated in the table below. When the animal was killed, after 4½ hours, a middle control loop was practically empty. The washings from it gave no biuret reaction in the filtrate obtained after slightly acidifying and heating. The casein loop contained a sticky mass soluble in hot water and yielding 0.312 gm. N, or 91 per cent of the amount introduced. That the proteid was unaltered sodium casein was made evident by its ready precipitation by acetic acid, and its solubility in dilute alkali, and further by its content of organic phosphorus. From the caseose loop only 4 mgm. of the 121 mgm. N introduced could be recovered in a similar manner.

¹ The coagulation-temperature of edestin is high.

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RECOVERED.

Casein	91 per cent.
Caseoses	3 "

SUMMARY OF RESULTS OF EXPERIMENTS ON THE ABSORPTION OF PROTEIDS FROM THE SMALL INTESTINE.

Number of experiment.	Substance used.	Weight of dog.	Nitrogen introduced.	Proteid equivalent of nitrogen.*	Volume of fluid introduced.	Length of loop used.	Duration of trial.	Proteid recovered.	Location of loop.
		kgm.	gram	grams	c.c.	cm.	hours	per cent	
IV	Edestin	12	0.412	2.19	28	..	4	68	
V	"	10	0.718	3.81	40	80	4½	89	30 cm. from cæcum.
VII	"	7.2	0.587	3.12	58	61	5½	69	30 cm. from pylorus; + control loop.
VIII	"	6.4	0.356	1.89	30	31	5½	75	10 cm. from pylorus; + 27 cm. control loop.
IX	"	11	0.366	1.95	24	26	5½	75	50 cm. from pylorus; + control loop.
VI	Edestin-proteose	9	0.397	..	31	44	5	none	44 cm. in upper third of gut.
VII	"	7.2	0.295	..	23	51	5½	17	15 cm. from cæcum.
VIII	"	6.4	0.251	..	34	34	5½	14	12 cm. from cæcum.
IX	Acid edestin	11	0.192	..	33	26	5½	84	20 cm. from cæcum.
XI	Casein	10	0.339	..	50	49	4½	69	3 cm. from cæcum.
XII	"	11.5	0.344	..	51	57	5	91	5 cm. from cæcum + 24 cm. control loop.
XII	Caseose	11.5	0.121	26	5	3.3	30 cm. from pylorus.

* Calculated from the nitrogen-content of the fluid by multiplication, using the appropriate factors.

These experiments indicate no essential differences in the fate of the two typical proteids edestin and casein within the intestine under conditions which practically exclude the normal digestive processes. Cohnheim¹ has found that the intestinal ferment erepsin slowly digests casein, although it is without action upon the other native proteids studied by him. In our experiments it is evident that little if any digestive change could be attributed to this enzyme in view of

¹ COHNHEIM: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 140.

the large quantities (70 to 90 per cent) of unaltered proteid recovered. Whether the proteoses underwent a cleavage to simpler compounds through the action of erepsin prior to their disappearance from the loops of intestine, or whether these readily diffusible digestion products were absorbed as such, cannot be ascertained from our observations. The protocols emphasize most strikingly, however, the importance of the preliminary digestive changes in facilitating the absorption of proteids.

In Experiment IV, in which a relatively large disappearance (32 per cent) of edestin from the gut was noted, the lymph was collected from the thoracic duct of the dog for a considerable period before and after the introduction of proteid into the loop. The lymph samples thus obtained were compared, with reference to their content of proteid, in the manner already described by one of us in a study of the paths of absorption for proteids.¹ In accordance with the earlier experience, no evidence of a passage of proteid into the lymph channels was obtained.

As evidence of the direct passage of unaltered proteids from the intestine into the tissue fluids, the instances of so-called "alimentary" albuminuria are frequently cited. By the use of the precipitin test, Ascoli² believes that he has demonstrated a passage of foreign proteid into both the blood and lymph of animals, the reaction being obtained in these fluids as well as the urine when excessive quantities of appropriate proteids are introduced. These statements have not remained unchallenged, however,³ nor is the "biological" test sufficiently developed, as yet, to give it unquestioned significance in absorption trials of this character.⁴ At any rate it has not given data of any quantitative value. We have made two attempts to find edestin (or its derivatives) in the urine of rabbits after introducing solutions of this proteid (in 0.75 per cent sodium carbonate solution) into the stomach through a sound. No proteid could be detected in either case. In one trial in which 17 gms. edestin in a volume of 100 c.c. were introduced, the animal was starved for two days and muzzled before and after the feeding, in order to keep the stomach

¹ MENDEL: This journal, 1899, ii, p. 137.

² Cf. ASCOLI and VIGAND: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 283, and earlier papers by ASCOLI in the *Münchener medicinische Wochenschrift*, 1902 and 1903.

³ See OPPENHEIMER: *Beiträge zur chemischen Physiologie*, 1904, iv, p. 265.

⁴ Cf. MOLL: *Beiträge zur chemischen Physiologie*, 1904, iv, p. 578.

empty by preventing the ingestion of the fæces.¹ It is not unlikely that the edestin, being precipitated in the stomach, is thus prevented from being absorbed before it has undergone digestive changes. But even in the event of a considerable absorption of unchanged proteid, we could not expect to detect it subsequently in the urine, in the light of our later experiments in which this proteid was introduced in considerable quantity directly into the circulation without being excreted again as such.

THE ABSORPTION OF NATIVE PROTEIDS FROM THE PERITONEAL CAVITY.

The studies on absorption from the peritoneal cavity have acquired additional importance in view of MacCallum's denial of the existence of open communications between the peritoneum and the lymphatics, and likewise of the idea that the peritoneal cavity forms a part of the lymphatic system.² Orlow³ and Hamburger⁴ both noted an absorption of blood-serum and ascites fluid to some extent from the cavity. The difficulties of obtaining anything more than approximate quantitative data in measuring the residual proteid in the peritoneal spaces are apparent, especially when the possibility of an exudation into the latter is considered. Nevertheless, we have made a few experiments with vegetable proteids on cats and rabbits, and have seen these substances disappear in not inconsiderable quantity. Special attention was directed to the possibility of an excretion of proteid in the urine in these trials.

Methods.—Edestin from hemp-seed and excelsin from the Brazil-nut⁵ were used, each being obtainable in crystallized form. The former was dissolved in one-half to three-quarters per cent sodium carbonate solution; the excelsin in 0.9 per cent sodium chloride solution. The warm solutions were introduced during anæsthesia through a small opening in the abdominal wall which was immediately closed again. Only such animals as secreted a proteid-free urine before the experiment were used. The urine was collected after the peritoneal injection and carefully tested

¹ Cf. SWIRSKI: *Archiv für experimentelle Pathologie und Pharmakologie*, 1902, xlviii, p. 282.

² MACCALLUM: *Johns Hopkins Hospital bulletin*, 1903, xiv, p. 115.

³ ORLOW: *Archiv für die gesammte Physiologie*, 1895, lix, p. 170.

⁴ HAMBURGER: *Archiv für Physiologie*, 1895, p. 281.

⁵ Prepared by OSBORNE's method: *American chemical journal*, 1892, xiv, p. 662.

(heat; Heller's reaction; acetic acid and potassium ferrocyanide). When the peritoneal cavity was opened after varying intervals, the fluid had usually disappeared, while the unabsorbed foreign proteid remained deposited in curdy masses. Edestin thus recovered could be recrystallized. The estimation of the extent of absorption was made on the basis of the nitrogen content of the soluble proteid material recovered. The following table gives a summary of the more interesting observations:

SUMMARY OF OBSERVATIONS ON THE ABSORPTION OF VEGETABLE PROTEIDS FROM THE PERITONEAL CAVITY.

Substance used.	Animal used.	Volume of fluid introduced.	Duration of trial.	Proteid recovered.	Urine.	Remarks.
<small>grams</small> Edestin, 2.10	Rabbit VIII	<small>c.c.</small> 50	<small>hours</small> 18½	½ of globulin N	No coagulable proteid; trace of proteose.	
" 2.10	Cat I	50	24	Very little	No proteid whatever	Injection syringe used.
" 1.82	Dog II	44	4½	½ of globulin N	Do.	
" 1.90	Cat II	46	72	Little	Do.	No pus or congestion.
Excelsin, 0.96	Rabbit I	75	24	Some unabsorbed, not determined	Proteose (15%) and coagulable proteid.	
" 0.73	Rabbit II	50	18	Do.	Do.	Died.
" 1.53	Rabbit III	48	17	Do.	Do.	Died.
" 0.80	Rabbit V	50	24	Not killed	Proteid free.	

THE FATE OF FOREIGN (VEGETABLE) PROTEIDS INTRODUCED DIRECTLY INTO THE CIRCULATION.

The disappearance of a proteid from the intestine or any other cavity is no proof, necessarily, of its passage into the blood; it may be retained within the absorbing membrane. The question as to the capacity of the animal body to assimilate foreign proteids without the intervention of the digestive processes has been answered in various ways. Numerous experiments by Neumeister led him to announce a distinction between assimilable and non-assimilable proteids, according to their behavior when injected directly into the

circulation.¹ The non-assimilable group included those compounds (egg-albumin, casein, and others) which speedily reappeared in the urine, and thus apparently were eliminated like other foreign substances. Regarding the various blood sera, the reports have been most conflicting. But Friedenthal and Lewandowsky² showed that if toxic sera of different species are first heated to 60° C., they can readily be assimilated after intravenous injection. More significant from our standpoint is the investigation of Munk and Lewandowsky,³ demonstrating that the most diverse types of proteids can be assimilated in noteworthy quantities directly from the circulation, provided only that they are injected very slowly and the rate of their introduction is thus made comparable with that pertaining during absorption from the gut. Since our experiments were undertaken, Oppenheimer⁴ has reported the results of intraperitoneal and intravenous injections of egg-proteid and blood-serum in rabbits, and has introduced the term *parenteral* to refer to those modes of introduction which avoid the alimentary canal. Like the previous authors, he has found the serum to be very completely retained; the egg-proteid was utilized less completely, being in part eliminated again in the urine. Repeated injections appeared to establish a certain degree of tolerance.

Methods.—Our experiments involve the fate of the crystallized vegetable proteids edestin and excelsin introduced slowly into the jugular vein during ether anæsthesia. The edestin was dissolved in sodium carbonate solution of just sufficient strength (0.2–0.7 per cent) to prevent precipitation at body temperature. The content of edestin (containing 18.7 per cent N) varied from 4 to 12 per cent; while the excelsin, dissolved in 0.9 per cent sodium chloride solution slightly alkaline with sodium carbonate, varied in amount from 0.6 to 4 per cent. The urine of all animals used was carefully tested beforehand and found proteid-free. When coagulable proteid was present after the injections, it was estimated by analysis of the well-washed heat-coagulum by the Kjeldahl N-method. As will be noted in the protocols, after injection of excelsin, the urines frequently contained a proteose-like substance which could not be

¹ NEUMEISTER: Lehrbuch der physiologischen Chemie, 1897, p. 301, contains a brief review of the literature.

² FRIEDENTHAL and LEWANDOWSKY: Archiv für Physiologie, 1899, p. 531.

³ MUNK and LEWANDOWSKY: Archiv für Physiologie, 1899, Supplementband, p. 73.

⁴ OPPENHEIMER: Beiträge zur chemischen Physiologie, 1904, iv, p. 263.

SUMMARY OF EXPERIMENTS IN WHICH VEGETABLE PROTEIDS
WERE INJECTED DIRECTLY INTO THE CIRCULATION.

Substance used.	Animal.	Volume of fluid injected.	Proteid per kilo body-weight.	Duration of injection.	Observations on the urine.	Conditions after recovery.
grams		c.c.	grams	min.		
Edestin, 3.18	Cat IV	78	0.62	78	Trace of coagulable proteid; no proteose	Depression.
" 1.08	" Va	20	0.54	15	No proteid whatever	Not abnormal.
" 1.08	" Vb	20	0.54	20	Do.	Do.
" 1.89	" VI	35	0.72	25	Do.	Slight depression.
" 2.80	" VII	52	1.08	41	Do.	Do.
" 1.64	" VIII	26	0.55	23	Do.	Good recovery.
" 3.35	" IX	53	0.88	35	Do.	Much depression.
" 4.95	" Xa	70	0.90	70	5% of N recovered	Good.
" 3.24	" Xb	40	0.75	90	Proteid-free	Fair; weak pulse during injection; rapid recovery.
" 1.95	" XIb	20	0.97	30	Trace of coagulable proteid	Fair.
" 4.38	" XII	36	0.88	40	2.8% N recovered	Bad; 60 c.c. blood had been taken.
" 3.68	" XIII	63	0.97	43	Proteid-free	30 c.c. blood had been taken.
" 2.33	" XV	30	0.87	..	Do.	Good.
" 2.72	" XVI	35	1.19	25	Little proteid	Great depression; had had kittens.
" 3.79	Dog Ia	60	1.05	55	Little coagulable proteid	Good.
" 2.30	" Ib	36	0.64	35	Do.	Good. 5 days after Experiment Ia.
" 5.35	" X	66	0.76	60	Do.	Good. Bile experiment.
" 7.78	" XIII	100	0.93	60	Proteid-free	Good.
" 3.27	" XIV	42	0.76	35	Do.	Do.
Excelsin, 0.42	Rabbit I	45	0.23	42	No coagulable proteid; proteoses. 49% of N recovered	Do.
" 0.67	" II	70	0.35	90	Do., 21% of N recovered	Do.
" 2.05	Dog II	139	0.32	55	Much proteose. Trace of coagulable proteid	Do.
" 1.45	Cat XIa	50	0.56	35	Protease. 5% of N recovered. No peptones	Do.

precipitated by coagulation, but responded to other proteid tests. Over twenty-five injection trials were made, the conditions as regard strength of solution, dose, etc., being widely varied. The skin wounds usually healed satisfactorily, and some of the animals were used for several experiments. Illustrative protocols follow.

Experiment 11.—A cat of 2.6 kilos received an injection of 50 c.c. of *excelsin* solution, containing 1.45 gms. proteid, in the course of thirty-five minutes. The cat recovered speedily, and the urine collected during the following day gave only the faintest tests for proteid by heat or Heller's test. The coagulation filtrate contained a proteose-like substance precipitable by zinc sulphate, yielding 0.0125 gm. N, or 4.7 per cent of the amount injected. There was no peptone present. On the second day the urine was free from all proteids. Ten days later the cat's weight had diminished to 2 kilos, owing to insufficient feeding. Twenty c.c. of *edestin* solution, containing 1.95 gms. proteid, were now injected in thirty minutes, respiration and pulse remaining fairly good. Urine collected two hours afterwards was proteid-free; the urine of the following two days contained very slight amounts of coagulable proteid, and none thereafter. The body-weight increased to 2.2 kilos.

These experiments demonstrate that in their behavior after direct introduction into the circulation in various species of animals, the typical vegetable proteids present no essential difference from that noted with proteids of animal origin. Their utilization—or properly speaking, their failure to reappear as such in the urine—was unusually good in practically all of the trials. The close resemblance between *edestin* and *excelsin* in chemical make-up is indicated by the differential analyses made according to Hausmann's method by Osborne and Harris,¹ namely:

	Total nitrogen.	Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	N in MgO precipitate.
Edestin (hemp-seed) . .	18.64	1.88	5.91	10.78	0.12
Excelsin (Brazil-nut) . .	18.30	1.48	5.91	10.97	0.17

Yet in distinction from *edestin*, the *excelsin* injections were followed by an elimination of a proteose-like substance, not coagulable by

¹ OSBORNE and HARRIS: Journal of the American Chemical Society, 1903, xxv, p. 348.

heat, but precipitable by zinc sulphate and other proteid precipitants, and giving a characteristic biuret reaction. This body was found in the urine of the dog, cat, and rabbit alike, and was also discovered after intraperitoneal excelsin injections. That it does not arise in the urine itself by transformation from excreted excelsin was shown by trials in which pure excelsin was found to retain its coagulability after solution in urine from the same animals. Whether — as seems likely — the product represents a derivative of the injected excelsin, and where the transformation occurs, cannot be told at present. We recall the related experience with various proteoses which have been found to undergo a digestive change prior to their elimination through the kidneys after intravenous injection.¹

With reference to *the immediate action of the intravenous injection*, symptoms comparable to those described by Brodie² as typical for serum-proteid injections were frequently observed, particularly in the cats. They consisted in a temporary inhibition of the heart's action and of respiration, and were occasionally of sufficient moment to cause death. The severity of the symptoms was usually increased with *the dose and rapidity of injection*. The sudden discharge of a few cubic centimetres of proteid solution into a vein may cause a profound effect, but if care is taken to prevent a repetition the heart slowly recovers. We usually made the injections with an intermittent flow. The appearance of more or less proteid in the urine seems to be determined less by the quantity injected than by the general condition of the animal. In agreement with Brodie's experience, we found the symptoms far less marked in dogs than in cats. In none of the experiments was there any evidence (hæmoglobinuria, choluria) of a destruction of erythrocytes.

Obviously paths of elimination other than the kidneys suggest themselves for the foreign proteids, as they have been emphasized by one of us for inorganic compounds.³ Gürber and Hallauer⁴ have quite recently considered the bile in this connection. In experiments on three rabbits with temporary fistulæ, they found a large part of the proteid in both *bile and urine* after injection of 2.7 gms. casein, although the properties of the proteid in the bile no longer exactly

¹ Cf. CHITTENDEN, MENDEL, and McDERMOTT: This journal, 1898, i, p. 275; CHITTENDEN, MENDEL, and HENDERSON: *Ibid.*, 1899, ii, p. 165.

² BRODIE: Journal of physiology, 1901, xxvi, p. 48.

³ MENDEL and THACHER: This journal, 1904, xi, p. 5.

⁴ GÜRBER and HALLAUER: Zeitschrift für Biologie, 1904, xlv, p. 372.

resembled those of the original casein. Remembering the very favorable results obtained by Munk and Lewandowsky in their careful injection experiments with casein (of which traces only were eliminated in the urine) one may well question whether the results obtained by Gürber and Hallauer were not due to unusual pathological disturbances, as in the experiments on the elimination of proteid in the bile reported by Brauer¹ and Pilzecker.² We have made observations on three dogs with temporary biliary fistulæ, to determine whether injected edestin is excreted through this channel. The results were negative in every case; an illustrative protocol of one experiment will suffice here. We have not investigated the possibility of an excretion of proteid into the intestine.

A dog of about 9 kilos body-weight was fed at 8 A. M. to promote the subsequent flow of bile; at 9.30, 60 mgms. morphine hydrochlorate were given subcutaneously, and later anæsthesia was induced with chloroform-ether mixture. At 10.30 a fistula was established and ox-bile injected into the rectum to accelerate the normal flow of bile. The normal bile contained considerable mucoid substance precipitable with acetic acid, but no other proteid precipitable by salt and heating or by alcohol. From 12.20 to 1.10, 90 c.c. of a solution containing 6 gms. of edestin were injected into the jugular vein. The bile collected until 4.15 behaved precisely like that collected before the injection. In another experiment, bile obtained after injection of 5 gms. of edestin into a small dog was dialyzed with the object of precipitating in crystalline form any edestin which might have been excreted. The results were likewise negative.

How is the injected proteid utilized?—From results obtained by means of the "biological" tests Sachs³ has reported that after transfusion of ox-blood into rabbits, it is in large part retained in the circulation for two or three days. We have attempted to ascertain whether the nitrogen equivalent of the injected proteid is eliminated in some form other than unutilized proteid. The method consisted in starving dogs until a constant daily N-elimination was attained, and then noting the effect of proteid injections on the composition of the urine. This plan was abandoned after two trials, because it was impossible to estimate the contribution of other factors such as anæsthesia,⁴ etc., to the increase in the eliminated nitrogenous com-

¹ BRAUER: *Zeitschrift für physiologische Chemie*, 1903, xl, p. 182.

² PILZECKER: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 157.

³ SACHS: *Archiv für Physiologie*, 1903, p. 495.

⁴ A slightly increased elimination of urinary nitrogen after ether anæsthesia has been reported by HAWK: *This journal*, 1904, x, p. xxxvii.

pounds after the injection, or to determine whether, and in what degree, the mere presence of the foreign proteid stimulated proteid katabolism. In each case the introduction of the proteid was followed by an increase in the nitrogen output considerably larger than could be accounted for by the substance injected. The urines were obtained by catheterization and were proteid-free in these trials. The protocols are of interest in emphasizing the uncertainty of conclusions drawn from a study of the nitrogen elimination as an index to parenteral absorption. This criticism applies to some of the earlier investigators by whom the method was employed.

NITROGENOUS METABOLISM AFTER INTRAVENOUS INJECTIONS
OF PROTEIDS.

	Day.	Body-weight.	Urine.	
			Volume.	N.
Dog XIII. (no food)		kgm.	c.c.	grams
	1	8.7	67	3.07
	2	8.5	54	2.56
	3	8.4	54	2.69
	4	8.4	70	2.38
	5	8.3	55	2.31
	6	8.2	130	2.51
	Intravenous injection of 7.78 gms. edestin (containing 1.46 gms. N) in 100 c.c. fluid during one hour.			
	7	7.9	155	7.09
	8	7.7	112	4.36
Dog XIV. (no food; 150 c.c. water daily)	9	7.5	64	3.01
	10	7.4	74	2.83
	1	4.6	78	2.03
	2	4.4	59	2.25
	Intravenous injection of 3.27 gms. edestin (containing 0.615 gm. N) in 42 c.c. fluid during 35 min.			
	3	4.2	96	3.37
	4	4.2	100	3.01
	5	4.1	56	2.44

Finally, we have attempted to follow the fate of the foreign globulins by estimating the relative proportions of the plasma proteids in cats at varying periods after the injections. Our method closely resembled that employed by Lewinski.¹ The results of a large number of experiments were so variable that we have been unable to draw any decisive inferences from them.

SUMMARY AND CONCLUSIONS.

Vegetable proteids (crystallized edestin from hemp-seed and excelsin from the Brazil-nut), slowly introduced in solution into the circulation of animals, can apparently be retained in the organism for the most part, even when the quantities introduced almost equal that of the globulins normally present in the blood. At any rate they are not eliminated unchanged in the urine (or in the bile, in the few experiments tried).

When solutions of vegetable proteids are injected too rapidly or in too great concentration, toxic symptoms, including an inhibition of the cardiac and respiratory activities, may be observed, especially in cats. This corresponds with observations of Brodie after serum-proteid injections in these animals.

The chemically similar proteids, edestin and excelsin, show slight differences in physiological action, a small amount of a proteose-like substance being found in the urine after intravenous or intraperitoneal (parenteral) introduction of excelsin, but not with edestin. The observation suggests the further possibility of applying chemico-biological reactions in distinguishing related proteids.

The vegetable proteids soon disappear in considerable part when introduced into the peritoneal cavity. That they reach the circulation is made probable in the case of excelsin at least, by the appearance of the typical urine proteose-body noted after direct intravenous injections. For the most part, however, the proteids do not reappear in the urine.

The unaltered proteids edestin and casein are absorbed to a very small extent, if at all, from portions of the living small intestine in which the ordinary digestive processes are excluded as far as possible. On the other hand, the proteoses and peptones obtained by peptic digestion of these proteids readily disappear from the intestine under the same conditions. It is not necessary to assume that in these

¹ LEWINSKI: *Archiv für die gesammte Physiologie*, 1903, c, p. 613.

cases they are first completely broken down by the intestinal enzyme erepsin; for casein (upon which erepsin can act) may remain unabsorbed. Dissolved edestin could be recovered in crystalline form, *i.e.*, unchanged after remaining in the intestine for several hours. The typical vegetable proteids show no marked differences from those of animal origin in their relation to the processes of metabolism.

The attempts to learn the fate of the foreign proteids retained in the system have been rather unsuccessful. It will be of interest to ascertain something further regarding their destination, and the exact mode of utilization which they undergo.

THE PRODUCTION OF CHOLIN FROM LECITHIN AND BRAIN-TISSUE.

By ISADOR H. CORIAT.

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INTRODUCTION.

MOST of the recent work on the autolysis of animal organs has related mainly to the detection of the hexon, purin, or pyrimidin bases, or to the estimation of the nitrogen content, either as ammonia, soluble nitrogen, in the heat coagulum, or in the zinc sulphate precipitate. Levene found that all the animal glands so far studied by him gave similar end-products on prolonged self-digestion. The work on brain-tissue is limited to the investigations of Levene¹ and the nitrogen determinations that were made sufficiently proved the presence of a probable intracellular, proteolytic enzyme. Austin² had previously produced oxalic acid in the digestion of brain-substance with pancreatin, but failed to detect it by heating lecithin in sealed tubes with barium hydrate. The most important of the decomposition-products of lecithin is cholin, which is found in the central nervous system, blood, and cerebrospinal fluid in those conditions where active degeneration is taking place. Studies in the production of cholin in various pathological states of the nervous system, especially in general paralysis and epilepsy, have been carried out by Mott and Halliburton,³ Donath,⁴ Wilson,⁵ and also by myself.⁶ The conclusions

¹ P. A. LEVENE and L. B. STOOKEY: *Journal of medical research*, 1903, x, p. 212.

² A. E. AUSTIN: *Boston medical and surgical journal*, 1901, clxv, p. 181.

³ W. D. HALLIBURTON: *The Croonian Lectures on the Chemical Side of Nervous Activity*, 1901; W. D. HALLIBURTON and F. W. MOTT: *Philosophical transactions*, 1899, p. 211; F. W. MOTT: *Archives of neurology*, 1903, ii, p. 858; W. D. HALLIBURTON and F. W. MOTT: *British medical journal*, 1899, p. 1082.

⁴ JULIUS DONATH: *Zeitschrift für physiologische Chemie*, 1903, xxxix, pp. 526-544; *Deutsche Zeitschrift für Nervenheilkunde*, 1904, xxvii, p. 71.

⁵ M. S. WILSON: *Revue neurologique*, 1904, p. 41.

⁶ I. H. CORIAT: *American journal of insanity*, 1903, lix, p. 393; 1904, lx, p. 733; *This journal*, 1903, x, p. 111.

arrived at show that the intracerebral injection or application of cholin produces severe paralytic effects, epileptiform and tetaniform seizures. It differs but little from neurin in its convulsive action, except that it is less toxic, but it shows some variations in its effect on blood-pressure. It is found in the cerebrospinal fluid, brain, and cord only in those conditions where there is active myelin decay, leading to a decomposition of the lecithin, and Donath has recently shown¹ that in these states there is a coincident increase in the phosphoric acid of the cerebrospinal fluid. This is easily explained if we remember that both cholin and glycerophosphoric acid pass into the fluid on the decomposition of lecithin, while the stearic acid combines with the glycerol, forms neutral fat, and accumulates beneath the neurilemma. This latter combination explains the osmic acid reaction. In two of his cases (tabes, Jacksonian epilepsy) Donath also detected lecithin, in one of which the myelin forms were present.

Gilson² failed to find cholin as the result of the action of weak sulphuric acid on lecithin, but found instead small quantities of glycerophosphoric acid, another phosphorus-containing compound (distearyl-glycerophosphoric acid), in still smaller quantities, and abundant amounts of free phosphoric acid. Dianconow,³ on shaking an ethereal solution of lecithin with sulphuric acid, found cholin-sulphate and distearyl-glycerophosphoric acid as the result of this reaction. Bókay⁴ found that lipase splits egg lecithin into glycerophosphoric acid, stearic acid, and neurin; but as none of these products appeared in the urine or fæces, he concluded that they must be absorbed and resynthesized. According to Hasebrock,⁵ glycerophosphoric acid, fatty acids, and cholin may be produced by the action of putrefactive bacteria on lecithin; the further action of these organisms splits the cholin into carbon dioxide, methane, ammonia, and trimethylamin, and these latter products may also be produced by cooking cholin with potassium or barium hydrate.

Vincent and Cramer⁶ find that normal ox blood, which contains no cholin, gives an organic platinum double salt consisting almost

¹ JULIUS DONATH: *Zeitschrift für physiologische Chemie*, 1904, xlii.

² E. GILSON: *Zeitschrift für physiologische Chemie*, xii, p. 585.

³ DIANCONOW: *Centralblatt für die medicinischen Wissenschaften*, 1868, p. 434.

⁴ A. BÓKAY: *Zeitschrift für physiologische Chemie*, 1877, i, p. 157.

⁵ K. HASEBROCK: *Zeitschrift für physiologische Chemie*, 1888, xii, p. 142.

⁶ S. VINCENT and W. CRAMER: *Journal of physiology*, 1903, xxx, p. 143.

entirely of potassium and ammonium platinochloride; while in a watery extract of nerve tissue, they find a platinum salt of dicholin anhydride. Gulewitsch¹ has also detected small quantities of cholin in the alcoholic extract of fresh ox brain, while neurin was absent; and Halliburton has found that normal human cerebrospinal fluid contains traces of cholin. These minute amounts, however, are confined to an exceedingly small number of crystals, and do not influence either the qualitative detection or quantitative estimation. The objections raised by Allen and French,² that these yellow octahedra of the double platinum salt may be either ammonium or potassium, will not stand critical inquiry from the standpoint of solubility, size, form, and arrangement of crystals, percentage of platinum, melting point, and the various reactions to alkaloidal reagents.

Mott and Barrett,³ in the examination of a degenerated cord from a case of hemiplegia, find a diminution of lecithin on the diseased side. Noll⁴ also in experimental section of the sciatic nerve, with consequent degeneration, found a decrease of phosphorus and of the alcoholic extract. In a series of experiments on rats, not yet published, I found that acute poisoning with strychnine, morphine, veratrine, cocaine, phosphorus, and alcohol is also effective in splitting up the lecithin, as cholin was found in both the brain and cord.

While these various decomposition-products, especially cholin, have been the subject of much attention, the factors concerned in the splitting up of the lecithin have remained unnoticed. The various lecithins from brain, egg, yeast, barley, and malt show a wide difference, not only in the fatty acid group, but in the phosphoric acid and its relation to the methyl content. The three methyl groups attached to nitrogen which form the cholin are more stable, and this is so well marked that lecithin can be quantitated by the estimation of these groups, according to the method of Herzig and Meyer. While this splitting can be accomplished in the laboratory by heating lecithin with barium hydrate, or by the action of lipase or putrefactive bacteria, yet in the human organism other factors must be sought. To this end, the recent work on autolysis and intracellular ferments is stimulating, and it was along these lines that the present research was conducted.

¹ W. GULEWITSCH: *Zeitschrift für physiologische Chemie*, 1899, xxvii, p. 81.

² R. W. ALLEN and H. FRENCH: *Journal of physiology* 1903, xxx, p. xxix.

³ F. W. MOTT and W. BARRETT: *Archives of neurology*, 1899, i, p. 346.

⁴ A. NOLL: *Zeitschrift für physiologische Chemie*, 1899, xxvii, pp. 4, 5.

METHODS.

The lecithin used was absolutely pure. It was isolated from calf brain, yolk of egg, and human brain (senile dementia), according to the method of Koch.¹ The pepsin and trypsin were very active preparations. The human brain material was obtained from a case of senile dementia, and was free from cholin, when analyzed according to the method detailed below.

The same method was used in all the experiments. After digestion had proceeded the required length of time, the solution was filtered (in case of the brain-material, by means of an exhaust filter), evaporated to dryness on the water bath, extracted with absolute alcohol, and again filtered. This was repeated twice, in order to insure the absence of potassium salts and any proteid. The operations were conducted at a temperature not exceeding 75° C., as lecithin decomposes at this point. If found necessary, the solution was decolorized with animal charcoal. To the final extract there was added an excess of a 5 per cent solution of platinum chloride in absolute alcohol. This resulting yellow precipitate was washed several times with absolute alcohol in order to remove all traces of the platinum salt, the residue dissolved in warm (40° C.) 15 per cent alcohol, and allowed to crystallize in a large watch-glass over calcium chloride. Although crystallization took several days, I failed to observe any separation of oleic acid, which, as Thudichum points out, takes place under these conditions with the platinum salt of lecithin and which, according to him, is due to a decomposition of the platinum compound. Cholin was designated as present, only if the large single and twin octahedral crystals were found, and if these were freely soluble in water and 15 per cent alcohol. The amount of platinum in this compound is 31.64 per cent, from which the absolute weight of the pure alkaloid was calculated. My compounds yielded 31.41 per cent platinum. In previous work along this line, I have further identified cholin by its positive reactions with alkaloidal reagents. The other platinum compounds liable to be formed are kephalin (3.595 per cent platinum), lecithin (10.2 per cent platinum), and neurin (33.6 per cent platinum); but only the platinum salt of cholin is soluble in water and 15 per cent alcohol. The other compounds do not dissolve in water or alcohol of any strength, but are precipitated from their ethereal solu-

¹ W. Koch: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, pp. 2, 3.

tions by absolute alcohol. Of these, neurin alone is liable to be mistaken for the cholin salt, but the solubility, percentage of platinum, shape of the crystals, and different melting point should readily distinguish it. Neurin crystallizes in single small octahedra, never in the large twin variety; it is soluble in hot water only with great difficulty, and the percentage of platinum is higher than that of cholin, and the melting point of the double salt is lower. Cholin melts at 240° – 241° C., neurin at 213° – 214° C. I have been unable to find neurin as a decomposition product of lecithin in any pathological cases, even when large quantities of cholin were present. A case of Huntington's chorea was carefully worked over for neurin, but none was found, although cholin was present in abundance. The platinum compound of lecithin is insoluble in alcohol of any strength. On account of the great differences in solubility, none of the above compounds except cholin, even if precipitated from a solution by platinum chloride, will be a source of error. Of the other decomposition-products of lecithin, neither the fatty acids nor glycerophosphoric acid yields a precipitate with platinum chloride. In the estimations, cerebrin also can easily be omitted, as it does not contain a methyl group and does not influence lecithin estimation, while kephalin contains only one methyl group.

In the protocols which follow, the amounts of cholin are expressed in terms of the absolute alkaloid, calculated from the platinum salt.

DETAILS OF EXPERIMENTS.

Series I.—*Pepsin and trypsin digestion, and decomposition of pure lecithins.*

In the following experiments 0.2 gram each of pure human, calf, and egg lecithin was used and allowed to digest in the different media for seventy-two hours at 38° – 40° C. The pepsin and trypsin were absolutely pure and very active preparations. For each digestion experiment, 0.2 gram of the enzyme was used; in the case of pepsin dissolved in 100 c.c. digestive hydrochloric acid, while the trypsin was dissolved in 100 c.c. 0.5 per cent sodium-carbonate solution. To this latter a few drops of chloroform were added to prevent putrefaction.

In the pepsin and trypsin experiments but little of the lecithin went into solution, whereas those in the neutral media (water) made a cloudy emulsion which remained uniform even on decomposition. An odor of putrefaction developed, and the solution became slightly acid, probably due to glycerophosphoric acid.

No. of experiment.	Lecithin.	Medium.	Cholin.
1	Human	Pepsin	^{gram} None.
2	Human	Trypsin	None.
3	Calf	Pepsin	None.
4	Calf	Trypsin	None.
5	Egg	Pepsin	None.
6	Egg	Trypsin	None.
7	Human	Water	0.0124
8	Egg	Water	0.0144
9	Calf	Water	0.0171

Series II. — Decomposition and autolysis. In the second series of experiments, fresh human brain-tissue, obtained from a case of senile dementia, was freed from blood and membrane; finely minced and accurately weighed quantities (10 grams) were distributed in neutral, acid, and alkaline media, and also subjected to the action of pepsin and trypsin, under the variations detailed below. The amounts of fluid in each case were 100 c.c., and the same quantities of pepsin and trypsin were used as in Series I. The temperature and time of digestion corresponded to the first series of experiments with one exception (Series V, Experiment 3).

No. of experiment.	Medium.	Cholin.	Remarks.
1	Normal salt solution	^{gram} 0.0513	Slight odor of putrefaction.
2	0.2% acetic acid solution . . .	None	No odor.
3	0.5% sodium carbonate solution	0.0547	Slight odor of putrefaction.

Series III. — Decomposition prevented by chloroform; autolysis alone.

No. of experiment.	Medium.	Cholin.	Remarks.
1	Normal salt solution	^{gram} 0.0192	No odor.
2	0.2% acetic acid solution . . .	None	No odor.
3	0.5% sodium-carbonate solution	0.0212	No odor.

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Series IV. — *The brain substance, after being placed in the various media, was boiled and cooled before autolysis was allowed to proceed. No chloroform was added; decomposition alone.*

No. of experiment.	Medium.	Cholin.	Remarks.
1	Normal salt solution	^{gram} 0.0219	Slight odor of putrefaction.
2	0.2% acetic acid solution . . .	None	No odor.
3	0.5% sodium-carbonate solution	0.0329	Slight odor of putrefaction.

Series V. — *The mixture was boiled and cooled as in Series IV; but chloroform was added to prevent decomposition, in order to show the negative action of putrefaction and autolysis.*

No. of experiment.	Medium.	Cholin.	Remarks.
1	Normal salt solution	^{gram} None	No odor.
2	0.2% acetic acid solution . . .	None	No odor.
3	0.5% sodium-carbonate solution	0.013	No odor.*

* Very slightly heated, and allowed to digest for 96 hours, in order to destroy only part of the enzyme and show the long-continued action of the small amount present.

Series VI. — *Action of pepsin and trypsin without previous heating of the brain-substance; combined action of the proteolytic enzymes and autolysis.*

No. of experiment.	Enzyme.	Cholin.	Remarks.
1	Pepsin	Absent	No odor.
2	Trypsin	Absent	No odor.

In Experiment 2, chloroform was added to prevent putrefaction.

Series VII. — *Same as Series VI, except that the brain-substance was heated and cooled before adding the solution containing the enzymes; isolated action of enzymes, without autolysis.*

No. of experiment.	Enzyme.	Cholin.	Remarks.
1	Pepsin	Absent	No odor.
2	Trypsin	Absent	No odor (chloroform added).

THEORETICAL AND ACTUAL YIELD OF CHOLIN.

In Table I, the theoretical yield of cholin required from pure lecithin, if all were split up, was calculated from the equation of the decomposition-products of lecithin. The yield from 1 gram of lecithin, if entirely decomposed, should be 0.1405 gram cholin. By splitting a sample (1 gram) of human lecithin with barium hydrate, 0.140 gram was obtained, which is within the limit of experimental error. The amounts of lecithin in brain-tissue were taken from Koch's figures of the analysis of the brain of an epileptic,¹ the average of the white matter (corpus callosum) and gray matter (prefrontal cortex) being 4.16 per cent.

TABLE III.

(Showing the amounts of cholin that should be theoretically produced if all the lecithin were split up, and the amounts practically found. Calculated from the formula of the decomposition-products of lecithin and from Koch's figures on the percentage of lecithin in brain-tissue.)

Series.	Number.	Amount calculated.	Amount found.
I	7	0.0281	0.0124
I	8	0.0281	0.0144
I	9	0.0281	0.0171
II	1	0.0584	0.0513
II	3	0.0584	0.0547
III	1	0.0584	0.0192
III	3	0.0584	0.0212
IV	1	0.0584	0.0219
IV	3	0.0584	0.0329
V	3	0.0584	0.013

¹ W. KOCH: This journal, 1904, xi, p. 303.

SUMMARY AND CONCLUSIONS.

1. The putrefaction of either human, calf, or egg lecithin, in a neutral medium, yields cholin, the reaction at the same time becoming acid. (Series I, Experiments 7, 8, 9.)

2. Human lecithin, by putrefaction alone, yields less cholin than either egg or calf lecithin, but the amount is about equal to the yield from brain-tissue during autolysis (Series III, Experiments 1, 3), but is less than the theoretical yield by calculation from the equation of the hydrolysis of lecithin.

3. The putrefaction of brain-tissue alone produces cholin and in a greater quantity than autolysis alone. (Series III, 1 and 3, and Series IV, 1 and 3.)

4. Lecithin is not split on prolonged contact with acids (hydrochloric and acetic, as in Series I, Experiments 1, 3, and 5, and Series II, 2; III, 2; IV, 2; V, 2; VI, 1; VII, 1).

5. Lecithin can be split by heating it with barium hydrate, and in this case the entire theoretical yield of cholin is obtained.

6. Neither pepsin nor trypsin is effective in splitting off the methyl group from any of these lecithins, so that cholin may be produced. (Series I, Experiments 1-6 inclusive.) Lipase, however, is capable of splitting lecithin.

7. Pepsin and trypsin not only fail to act on the lecithin of brain-tissue, but actually seem to inhibit or even destroy autolysis. (Series VI and VII.)

8. There is an enzyme present in brain-tissue, capable of splitting cholin from lecithin.

9. The enzyme acts only in neutral or slightly alkaline media, and the yield of cholin in the latter is greater than in the former. The enzyme is inactive in slightly acid media. Levene found that the autolysis of brain-tissue, so far as the proteolytic process was concerned, was favored by the presence of an acid and inhibited by an alkali. The lecithin-splitting enzyme appears to have a contrary action. In the body, the action of the enzyme is favored by the normal alkaline reaction of the nerve-substance and cerebrospinal fluid. The ease with which this enzyme acts on lecithin is probably explained by the fact that the lecithin in the central nervous system, as in the stroma of red blood-corpuscles, is not in a chemical combination, but in an emulsiform condition, and is therefore capable of mechanical solution. According to Koch, brain lecithin, which

has been a part of living tissues, gives a more perfect emulsion than egg lecithin, which is merely stored-up food material. These emulsions have many of the physical properties of living protoplasm in that precipitation of the emulsion takes place by divalent kations, and is prevented by univalent and trivalent kations. This I have also observed.

10. The enzyme can be destroyed by heating, and then, if the suspension of the brain-tissue be kept absolutely sterile, no cholin is produced (Series V, 1); if putrefaction is allowed to supervene, cholin will be formed in a greater quantity than by autolysis alone. (Series IV, 1 and 3.)

11. Prolonged action with antiseptic precautions, and with very slight preliminary heating, so as to destroy only part of the enzyme, produces a very small amount of cholin. (Series V, Experiment 3.)

12. As with all enzymes, there is an inhibitory influence of reaction-products which cannot be removed, and this explains the low percentage of cholin obtained.

13. Efforts to isolate this enzyme have so far been unsuccessful.

14. The cholin produced in the combined action of autolysis and putrefaction is nearly equal to the sum of each, when acting separately in similar media, and nearly approaches the theoretical amount which should be yielded by the percentage of lecithin in the weight of brain-tissue used.

15. The amounts of cholin actually produced, both by the decomposition of pure lecithin and in the autolysis of brain-tissue, is less than that theoretically required for the quantity of lecithin present, if all of it were split up (according to the equation of decomposition). The only approach to the theoretical amount is when lecithin is saponified with barium hydrate, or by the combined action of putrefaction and autolysis.

FURTHER EXPERIMENTS ON THE HÆMOLYSINO-
GENIC AND AGGLUTININOGENIC ACTION OF
LAKED CORPUSCLES.

BY G. N. STEWART.

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IN a recent paper¹ I investigated the distribution of the hæmoly-sinogens and agglutininogens in the stromata and extracorpuseular liquid of colored corpuscles laked in various ways, injecting into one series of animals the washed ghosts, and into another series the liquid of foreign corpuscles, and testing the sera of the injected animals, after a suitable interval, as regards their power of agglutinating and laking corpuscles of the same kind as those injected. In the preparation of the material for injection five methods of laking were employed (heat, freezing and thawing, foreign serum, saponin, and water). Some of these methods, in accordance with the classification adopted in a previous communication² may be considered "violent," since they can be shown to affect the stroma more profoundly than the others, which we may designate by contrast as "mild." The general result of that investigation was that although after most methods of laking the stroma was more strongly agglutininogenic than hæmolyisinogenic, and the liquid the reverse, no complete separation from the corpuscle either of agglutininogen or of hæmolyisinogen could be effected by any of the methods of laking employed. In other words, the stromata of the laked corpuscles still possessed, in general, both agglutininogenic and hæmolyisinogenic powers, and both of these powers had also been acquired by the extracorpuseular liquid. This result is opposed to the conclusion of Nolf,³ who, working with water-laked corpuscles, convinced himself that the agglutininogenic substance was entirely contained in the stroma, and the hæmolyisinogenic entirely in the liquid. Ford and Halsey⁴ also,

¹ STEWART: This journal, 1904, xi, p. 250.

² STEWART: Journal of physiology, 1899, xxiv, p. 211.

³ NOLF: Annales de l'Institut Pasteur, 1900, xiv, p. 297.

⁴ FORD and HALSEY: Journal of medical research, 1904, xi, p. 403.

after carefully investigating the action of water-laked corpuscles, and, for the sake of comparison, following in all its details the procedure recommended by Nolf, have been unable to confirm his statement. In all their experiments, agglutination and hæmolysis were both caused by any serum which caused one of them. In some cases where the serum had no hæmolytic effect, but nevertheless produced agglutination, they proved that the absence of hæmolysis was due not to the want of the specific intermediary body, but to the lack of complement.

Here I desire to record the results of some further experiments which were not completed in time to be included in the previous paper. They were made on four fresh rabbits and two fresh guinea-pigs. In addition, observations were carried out on two of the

TABLE I.

Material injected.	Animal.	Agglutination.	Laking.
Liquid of water-laked dog's corpuscles, separated by centrifuge in first two injections, in the third filtered through clay	Rabbit F	Good	Some.
Ghosts of water-laked dog's corpuscles (3 injections)	Rabbit G	Good	Some.
Liquid of dog's corpuscles laked by freezing and thawing, filtered through clay (3 injections) .	Rabbit J	None	None.
Ghosts of dog's corpuscles laked by freezing and thawing (2 injections)	Rabbit K	Fair	None.
Liquid of rabbit's corpuscles laked by dog's serum filtered through clay (2 injections) .	Guinea-pig R	None	None.
Ghosts of rabbit's corpuscles laked by dog's serum (2 injections)	Guinea-pig S	None	Fair.

rabbits and one of the guinea-pigs used in the previous work, to determine for how long a period the modification in the serum persisted. The two fresh guinea-pigs received respectively washed rabbit's ghosts laked by dog's serum, and the liquid separated from the ghosts by filtration through unglazed porcelain. Two of the rabbits received respectively the ghosts and the liquid of water-laked dog's corpuscles, the liquid for the first two injections being separated from the ghosts by prolonged centrifugalization, but in the third by filtering through clay. The other two rabbits were injected respectively with the liquid and the ghosts of dog's corpuscles laked by freezing and thawing, the liquid being filtered through clay. All these animals received at least two injections. Some of them

received three. The results of the examination of the sera are summarized in Table I.

The moderate laking caused by the sera of rabbits F and G was shown, by activating dog's corpuscles with them at 0° C., to be due to a specific intermediary body. The attempt made to demonstrate that the same was true for the laking action of the serum of Guinea-pig S on rabbit's corpuscles was not successful. The results for the liquid and ghosts of the water-laked blood agree substantially with those of the previous paper. While both cause the production of serum with specific hæmolytic as well as agglutinating power, it is the agglutinating power which preponderates. The same preponderance which was noted in the former paper, in the case of guinea-pigs injected with the stromata of rabbit's corpuscles, laked by freezing and thawing, is even more evident in the case of Rabbit K, which received the ghosts of dog's corpuscles laked in the same way. Here the serum had no noticeable hæmolytic effect, but caused fair agglutination of dog's corpuscles. The want of laking power was apparently not due to the absence of complement, since the addition of dog's serum did not alter the result. For the stromata of rabbit's corpuscles laked by foreign (dog's) serum, and injected into guinea-pigs, the negative result as regards agglutination supplements the table in the previous paper where observations on this point were missing. I do not propose at present to discuss the significance of this negative result. Although there was but a single experiment, one cannot help feeling some confidence in it since agglutination is in general very easily and strikingly demonstrated with immune sera produced by the injection of the stromata of corpuscles laked in other ways. Although in the two injections which this guinea-pig (S) received, a relatively large quantity of ghosts (corresponding to 18 c.c. of blood) was introduced, a larger number of injections at shorter intervals might possibly give a different result.

The most interesting point shown in the Table is the absence of both specific hæmolysin and agglutinin from the serum of Rabbit J, which received the liquid of dog's corpuscles laked by freezing and thawing, and from the serum of Guinea-pig R, which received the liquid of rabbit's corpuscles laked by dog's serum, both liquids having been filtered through a pot of porous earthenware before injection. In the last paper it was stated that the injection of the liquid of corpuscles laked by freezing and thawing confers upon the serum of the animal into which it is injected the power of agglutinating and laking

the corresponding corpuscles. Here the liquid was separated from the stromata by the centrifuge and was not filtered. Apparently, then, the porous clay prevents the agglutininogens and hæmolysins from passing through. It is, of course, possible that a small amount does pass through the filter, and that a larger number of injections might give a positive result. The difference in the action of the centrifugalized and filtered liquids could be explained on the assumption that the former still contained, even after prolonged and repeated centrifugalization, a sufficient remnant of stromata to cause distinct agglutininogenic and hæmolysinogenic reactions. But this is by no means likely, as the result of centrifugalization was controlled by the microscope, and no liquid was injected which was seen to contain many ghosts. It is more probable that in laking some of the agglutininogenic and hæmolysinogenic substances are extruded from the corpuscles in colloidal solution, or contained in those granules which are commonly seen in laked blood, and that in either case the pores of the clay refuse them passage. The agglutinin in typhoid serum, it is said, is removed by filtration through porcelain. The pot used in these experiments was shown (see protocols) to remove the greater part of the hæmolytic power of normal dog's serum for rabbit's corpuscles. This was apparently due in part to the removal of complement, but not entirely, since the hæmolytic power could not be fully restored by the addition of rabbit's serum. The agglutinating power of the unfiltered serum was relatively feeble, but it was distinctly diminished by filtration. The fact that the agglutininogens and hæmolysins in the liquid of laked corpuscles are apparently also incapable of passing through such a filter, strengthens the view that the substances in blood which when injected into an animal give rise to the formation of agglutinins and hæmolysins are nearly akin to the bodies whose production they cause. Since the hæmoglobin passed through the pot used in these experiments, the negative result confirms the statement of Ford and Halsey¹ that solutions of pure hæmoglobin do not lead to the formation of hæmolytic or agglutinating substances.

In Table II are given the results of the further examination of the sera of Rabbit A and Guinea-pigs F and J' already reported on in the former paper. These animals were kept alive with the view of testing how long the specific alterations in the serum persist.

¹ FORD and HALSEY: *Loc. cit.*

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The serum of Guinea-pig J', which, 21 days after the last injection of the stromata of heat-laked rabbit's corpuscles, gave good agglutination of rabbit's corpuscles, caused, 107 days after the last injection, only very moderate agglutination, although still very fair laking in comparison with that produced by normal guinea-pig's serum. The serum of Guinea-pig F, which, 41 days after a single injection of normal rabbit's corpuscles, caused good agglutination and laking of rabbit's corpuscles, still after 180 days produced fair agglutination and laking, in comparison with the serum of the practically normal Guinea-pig R (Table I), although distinctly less than before. Evidence was obtained of the presence of specific intermediary body in the serum of F after 180 days. The serum of Rabbit A, which, 18 days after the last injection of dog's formaldehyde-fixed corpuscles,

TABLE II.

Material injected.	Animal.	Days after last injection.	Agglutination.	Laking.
Stromata of heat-laked rabbit's corpuscles (two injections) .	{ Guinea-pig J'	107	Some Good	Very fair.
		21		Good.
Normal rabbit's corpuscles (one injection)	{ Guinea-pig F	180	Fair	Fair.
		41	Good	Strong.
Dog's formaldehyde-fixed corpuscles (two injections) . .	{ Rabbit A	148	Slight	Very slight.
		18	Good	Fair.

gave good agglutination and fair laking of dog's corpuscles, caused, 148 days after the last injection, only a little agglutination and very little laking. The agglutination, however, was better marked than the laking, just as when the first test was made. So far as one can say from a determination of two points on the curve, the decline in agglutinating, ran parallel to the decline in lytic power, the latter being practically extinguished by the time of the second test. There was no evidence that the feebleness of the hæmolytic action was due to deficiency of complement since the addition of normal dog's serum did not increase it.

SUMMARY.

The results of the previous paper in regard to the agglutininogenic and hæmolytinogenic power of the stromata and liquid of water-laked corpuscles receive additional support. Both cause the pro-

duction of sera with specific hæmolytic and agglutinating power, the latter being most marked, as is also the case with the serum obtained after the injection of stromata laked by freezing and thawing. Filtration through porous earthenware appears to remove the agglutininogens and hæmolysinogens from the liquid of corpuscles laked by freezing and thawing and by foreign serum.

Small Rabbit F. *May 6, May 16, and June 15.*—Injected liquid from water-laked dog's corpuscles corresponding, respectively, to 15 c.c., 18 c.c., and 15 c.c. of blood. For the first two injections, the liquid was centrifugalized, after the addition of sodium chloride, till practically no ghosts could be seen with the microscope. For the third injection it was filtered through clay.

July 9.—Got serum (F). Made the following experiments:

- (1) S* + 0.2 c.c. F. 40 m (40°),* complete L.*
- (2) S + 0.1 c.c. F. 40 m (40°), good A.* 55 m (40°), L not yet complete; 14 h (r),* nearly complete.
- (3) S + 0.05 c.c. F. 40 m (40°), little L. 115 m (40°) and 14 h (r), some L, but not much. 22 m (40°), marked A. Good A in few minutes at 40°.

Repeated with S'. 15 m (40°), no L in any, but good A in all. 43 m (40°), slight L in (1), none in (2) and (3); 14 h (r), no L in (3), very little in (2), some L in (1), but not half laked. In all L is much less than in the corresponding experiments with S.

Repeated (1), (2), and (3) with S and F serum which had been heated to 58° for 20 minutes; 60 m (40°), no L; 14 h (r), slight L; 8 m (40°), excellent A in (1), and good A in (2) and (3).

Repeated (1), (2), and (3) with S and heated F serum diluted with 9 times its volume of NaCl solution; 4 h (40°), no A or L in any.

Repeated (1), (2), and (3) with S and normal rabbit's serum, and with S and normal rabbit's serum previously heated to 58°; 2½ h (40°), and 18 h (0°), no A or L in any.

* In the protocols, S means 0.5 c.c. of a 5 per cent suspension of washed dog's corpuscles where rabbit's serum was being tested, and of washed rabbit's corpuscles where guinea-pig's serum was being tested. S' means 0.5 c.c. of a 10 per cent dilution of the entire blood of dog or rabbit, respectively. Where smaller quantities of serum than 0.1 c.c. were to be added to 0.5 c.c. of suspension, the requisite dilution was obtained by adding more than 0.5 c.c. of suspension to 0.1 c.c. of serum; but, for simplicity in the protocols, the amount of serum is stated as if it were always added to 0.5 c.c. 40 m (40°) means 40 minutes at 40° C. 14 h (r) means 14 hours at room-temperature. 14 h (0°) would mean 14 hours in the ice-chest. A stands for agglutination, L, for laking.

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Mixed 1 c.c. of a sediment of washed dog's corpuscles with 1 c.c. F serum, both previously cooled to 0° C.; kept 2½ hours at 0°, then took off the serum (α), washed the sediment twice in the centrifuge, and made a 5 per cent suspension of it (β). Performed the following experiments:

- (4) S + 0.2 c.c. α + 0.1 c.c. NaCl solution.
- (5) S + 0.1 c.c. α + 0.1 c.c. NaCl solution.
- (6) S + 0.2 c.c. α + 0.1 c.c. normal dog's serum.
- (7) S + 0.1 c.c. α + 0.1 c.c. normal dog's serum.
- (8) S + 0.1 c.c. α + 0.1 c.c. heated F serum.
- (9) S + 0.1 c.c. normal rabbit's serum + 0.1 c.c. heated F serum.

After 50 m (40°) and 18 h (0°), fair L in (8), better than in any of the Experiments (4) to (14), except (12). Some A in (4), (6), and (8). Good A in (9). Fair L in (9). Slight L in (4) and (5). No L in (6) and (7).

- (10) 0.5 c.c. β + 0.1 c.c. normal dog's serum.
- (11) 0.5 c.c. β + 0.2 c.c. normal dog's serum.
- (12) 0.5 c.c. β + 0.2 c.c. normal rabbit's serum.
- (13) 0.5 c.c. β + 0.2 c.c. serum α .
- (14) 0.5 c.c. β + 0.2 c.c. NaCl solution.

After 50 m (40°), 18 h (0°), fair A in (12) and (13); some A in the others. Good L in (12), much the best of all the Experiments (4) to (14). Slight L in (10), and some in (13). Little or no L in (11). No L in (14).

Small Rabbit G. *May 7, May 16, and June 17.* — Injected the stromata of dog's water-laked corpuscles corresponding, respectively, to 20 c.c., 20 c.c., and 15 c.c. of blood.

June 25. — Got serum (G). Heated some to 57° for 20 minutes.

- (1) S + 0.2 c.c. G. 20 m (36°), distinct L. 60 m (36°), complete L., preceded by A.
- (2) S + 0.1 c.c. G. 60 m (36°), good A, comparatively little L. 2 h (36°), very fair L.
- (3) S + 0.1 c.c. NaCl solution. 90 m (36°), no A or L.
- (4) S + 0.2 c.c. G (heated). 10 m (36°), marked A. 90 m (36°), no L.

Mixed a sediment of dog's corpuscles (at 0°) with G serum (at 0°), and left 1½ hours at 0°. The corpuscles were well agglutinated. Now separated the serum (α), washed the sediment, and made a 5 per cent suspension of it (β).

- (5) 0.5 c.c. β + 0.2 c.c. dog's serum. Pretty good A. 1½ h (36°), and 1 h (r), fair L, but not complete.
- (6) S + 0.1 c.c. G + 0.1 c.c. dog's serum.
- (7) S + 0.1 c.c. α + 0.1 c.c. dog's serum.
- (8) S + 0.2 c.c. NaCl solution.

- (9) S + 0.1 c.c. heated G serum + 0.1 c.c. dog's serum.
 (10) S + 0.1 c.c. heated G serum + 0.1 c.c. a + 0.1 c.c. dog's serum. 50 m (36°), 1 h (r). Fair L.
 (11) S + 0.1 c.c. heated G serum + 0.1 c.c. a. 50 m (36°), 1 h (r). Fair L.

Agglutination is absent or inconspicuous in Experiments (5) to (11). Little if any L in Experiments (6) to (9) in 1½ h (36°) and 1 h (r).

Small Rabbit J. *May 29, May 30, and June 25.*—Injected liquid from dog's corpuscles laked by freezing and thawing, corresponding, respectively, to 15 c.c., 10 c.c., and 12 c.c. of blood. The liquid was filtered through clay.

July 16.—Got serum (J). Heated some of it to 58° for 20 minutes.

- | | |
|---------------------------------------------|------------------------------------------------------------------------------------|
| (1) S + 0.1 c.c. J. | (8) S + 0.1 c.c. heated J + 0.1 c.c. dog's serum. |
| (2) S + 0.05 c.c. J. | |
| (3) S + 0.1 c.c. heated J. | (9) S + 0.05 c.c. heated J + 0.1 c.c. dog's serum. |
| (4) S + 0.05 c.c. heated J. | |
| (5) S + 0.1 c.c. NaCl solution. | (10) S + 0.2 c.c. J. |
| (6) S + 0.1 c.c. J + 0.1 c.c. dog's serum. | (11) S + 0.2 c.c. J + 0.2 c.c. dog's serum. |
| (7) S + 0.05 c.c. J + 0.1 c.c. dog's serum. | (12) 0.5 c.c. of a 5 % suspension of J's washed corpuscles + 0.1 c.c. dog's serum. |

In 1½ h (40°) no A or L in any of Experiments (1) to (11). Even after 15 h (40°), and 24 h (0°) very little L. In (12) complete L in 20 minutes, and no doubt sooner.

Rabbit K. *May 30 and July 22.*—Injected all the washed stromata which could be separated from the corpuscles of 20 c.c. and 22 c.c., respectively, of dog's blood laked by freezing and thawing.

July 24.—Got serum (K). Heated some to 57° for 20 minutes.

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|-----------------------------------------|---------------------------------------------------|
| (1) S + 0.1 c.c. K. | (5) S + 0.1 c.c. heated K + 0.1 c.c. dog's serum. |
| (2) S + 0.05 c.c. K. | |
| (3) S + 0.1 c.c. NaCl solution. | (6) S + 0.1 c.c. heated K + 0.2 c.c. dog's serum. |
| (4) S + 0.1 c.c. heated K. | |
| (7) S + 0.025 K. | |
| (8) S + 0.025 K + 0.4 c.c. dog's serum. | |

No L after 2 hours and 40 minutes at 40°, and 12 hours at 0°. Fair but not strong A in all except (3); seems strongest in (4).

Guinea-pig F.—Injected washed rabbit's corpuscles on Jan. 20.

July 19.—Got serum (F). Heated some to 58° for 15 minutes.

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|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| (1) S + 0.1 c.c. F. 25 m (40°), almost complete L, preceded by A. L began even at room-temperature immediately after addition of serum. Half this amount of F serum also caused fair laking (see protocol of Guinea-pig R). | |
| (2) S + 0.1 c.c. heated F. | (4) S + 0.1 c.c. heated F + 0.1 c.c. rabbit's serum. |
| (3) S + 0.05 c.c. heated F. | |
| (5) S + 0.05 c.c. heated F + 0.1 c.c. rabbit's serum. | |

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After 4 h (40°) very little L in (2) to (5), but fair A in all.

Added F serum at 0° to washed rabbit's corpuscles at 0°, and kept at 0° for 3 hours and 20 minutes. A little L, fair A. Now separated the serum and diluted it with 4 volumes NaCl solution. Call the dilute serum α . Washed the sediment and made a 5 per cent suspension (β).

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|-------------------------------------------------|-----------------------------------------------------|
| (6) S + 0.5 c.c. α . | (11) S + 0.6 c.c. NaCl solution. |
| (7) S + 0.25 c.c. α . | (12) S + 0.25 c.c. α + 0.05 c.c. heated F. |
| (8) S + 0.5 c.c. NaCl solution. | (13) S + 0.3 c.c. NaCl solution. |
| (9) S + 0.25 c.c. NaCl solution. | (14) 0.5 c.c. of β + 0.1 c.c. NaCl solution. |
| (10) S + 0.5 c.c. α + 0.1 c.c. heated F. | (15) 0.5 c.c. of β + 0.1 c.c. rabbit's serum. |

After 45 m (40°), fair L in (10) and (12), none in the others.

After 90 m (40°), nearly complete L in (10); good L, but not complete, in (12); none in the others. Fair A in (14) and (15). Same after 3 h (0°).

Guinea-pig J', 850 grams. *Jan. 26 and April 8.*—Injected the heat-laked stromata of rabbit's corpuscles corresponding, respectively, to 7 c.c. and 3.5 c.c. of blood.

July 24.—Got serum (J'). Heated some to 57° for 15 minutes, and 58° for 5 minutes.

- (1) S + 0.1 c.c. J'. 15 m (40°), good L, not quite complete. 85 m (40°), complete L. No doubt complete earlier.
- (2) S + 0.05 c.c. J'. 15 m (40°), very fair L, and some A. 30 m (40°), L is nearly as great as in (5) at same time. 85 m (40°), good L, though less than in (5). Some A. 3 h (40°), L still incomplete.
- (3) S + 0.1 c.c. heated J'. 85 m (40°), no L; some A, but not marked.
- (4) S + 0.1 c.c. J' + 0.1 c.c. rabbit's serum. 10 m (40°), complete L.
- (5) S + 0.05 c.c. J' + 0.05 c.c. rabbit's serum. 15 m (40°), some L; 80 m (40°), almost complete L; 3 h (40°), complete L. Some A, but not strong.
- (6) S + 0.025 c.c. J'. 1 h (40°), some L; 2½ h (40°), fair L. Some A.
- (7) S + 0.025 c.c. J' + 0.05 c.c. rabbit's serum. 1 h (40°), some L, but less than in (6). 2½ h (40°), L about same as in (6), and also A.
- (8) S + 0.025 c.c. heated J'. 2½ h (40°), A as in (6). No L.
- (9) S + 0.05 c.c. NaCl solution. No L or A.
- (10) S + 0.1 c.c. heated J' + 0.1 c.c. rabbit's serum. 40 m (40°), some L. 55 m (40°), good L; in one hour more, nearly complete L.
- (11) S + 0.1 c.c. heated J' + 0.2 c.c. rabbit's serum. 75 m (40°), complete L.
- (12) S + 0.025 c.c. J' + 0.1 c.c. rabbit's serum. 2 h (40°) and 12 h (0°), no L; fair A. A is not conspicuous in any of Experiments (1) to (12).

Guinea-pig R, 750 grams. *June 4 and June 25.*—Injected the liquid from rabbit's corpuscles laked by dog's serum corresponding, respectively, to 9 c.c. and 6 c.c. of blood.

July 18.—Got serum (R). Heated some to 57°. Made the following experiments to test the laking and agglutinating power of the serum and to compare it with the serum of Guinea-pig F.

- (1) S + 0.1 c.c. R. 40 m (40°), no L or A. After 11 h (0°), some L, but much less than in (3).
- (2) S + 0.05 c.c. R. 40 m (40°) and 11 h (0°), only the very slightest L, and little, if any, A.
- (3) S + 0.1 c.c. F. 25 m (40°), almost complete L, preceded by A.
- (4) S + 0.05 c.c. F. 25 m (40°), fair L and A. 40 m (40°) and 11 h (0°), fair L, better than in (1), and good A.

Made a 10 per cent dilution of normal rabbit's blood (S').

- (5) S' + 0.1 c.c. R. 25 m (40°), no L; very little after 11 h (0°), distinctly less than in (1). Little or no A.
- (6) S' + 0.05 c.c. R. No L or A.
- (7) S' + 0.025 c.c. R. No L or A.
- (8) S' + 0.1 c.c. F. 14 m (40°), complete L.
- (9) S' + 0.05 c.c. F. 14 m (40°), some L. 25 m (40°) and 11 h (0°), fair L, but less than in (4). Good A.
- (10) S + 0.025 c.c. F. Fair L, but less than in (9) under same conditions. Good A, much better than in (2).

Guinea-pig S, 800 grams. *June 3 and June 26.* Injected the stromata of rabbit's corpuscles laked by dog's serum corresponding, respectively, to 12 c.c. and 6 c.c. of blood.

June 28. — Got serum (Ser). Heated some to 57° for 20 minutes.

- | | |
|-------------------------------------------------|---------------------------------|
| (1) S + 0.1 c.c. Ser. | (3) S + 0.1 c.c. NaCl solution. |
| (2) S + 0.1 c.c. Ser + 0.1 c.c. rabbit's serum. | (4) S + 0.05 c.c. Ser. |
| | (5) S + 0.1 c.c. heated Ser. |

In (1) some L in 12 m (36°), but by no means complete even after 45 m (36°) and 12 h (r). In (2) good L in 20 m (36°), not quite complete. Nearly complete in 80 m (36°), and distinctly better than in (1).

In (4) very slight L in 80 m (36°), although more than in control (3).

After 12 h (0°) slight but distinct L in (4). No L in (5). A is absent or inconspicuous in all.

Added to a sediment of washed rabbit's corpuscles at 0° serum of Guinea-pig S at 0°, and left at 0° for 1½ hours. Separated the serum (α) and made a 5 per cent suspension of the washed corpuscles (β).

- | | |
|-------------------------------------------------|----------------------------------------------------------------------|
| (6) 0.5 c.c. β + 0.2 c.c. rabbit's serum. | (11) S + 0.1 c.c. heated Ser + 0.1 c.c. rabbit's serum. |
| (7) 0.5 c.c. β + 0.2 c.c. NaCl solution. | |
| (8) S + 0.1 c.c. Ser + 0.1 c.c. rabbit's serum. | (12) S + 0.1 c.c. α + 0.1 c.c. heated Ser + 0.1 c.c. rabbit's serum. |
| (9) S + 0.1 c.c. α + 0.1 c.c. rabbit's serum. | (13) S + 0.1 c.c. α + 0.05 heated Ser. |
| (10) S + 0.2 c.c. NaCl solution. | (14) 0.5 c.c. β + 0.2 c.c. α. |
| | (15) 0.5 c.c. β + 0.2 c.c. rabbit's serum. |
| | (16) 0.5 c.c. β + 0.2 c.c. NaCl solution. |

After 3½ h (36°), no L in any of Experiments (6) to (16), except in (8), where L is very fair, though not complete, and in (12), where there is a trace of L. A is absent or inconspicuous in all, except in (6) and (7).

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Filtered serum. — Filtered dog's serum through the clay pot, throwing away the first part of filtrate. Call unfiltered serum D and filtered D'. Heated some of D and D' to 57° for 25 minutes. During the heating nearly all the blood-pigment went out of solution in D', while no change occurred in D. The spectrum of the heated D' showed feeble oxyhæmoglobin bands. Made a 5 per cent suspension of washed rabbit's corpuscles (S) and a 10 per cent dilution of rabbit's blood (S').

- (1) S' + 0.05 c.c. D. 40 m (40°), partial L.; 2½ h (40°), very fair L.
- (2) S' + 0.1 c.c. D. 40 m (40°), complete L.
- (3) S' + 0.05 c.c. D'. 5 h (40°), no L.
- (4) S' + 0.1 c.c. D'. 2½ h (40°), no L. 5 h (40°), slight but distinct L.

Repeated (1) to (4) with S instead of S'. Practically the same result.

Repeated (1) to (4) with S instead of S', and heated D and D' instead of unheated. No L in any. Fair A in (2), none in the rest.

- (5) S + 0.1 c.c. heated D + 0.2 c.c. D'. 70 m (40°), some L. 90 m (40°), 17 h (0°), fair A, very fair L (quite half laked).
- (6) S + 0.1 c.c. heated D + 0.2 c.c. rabbit's serum. } 1½ h (40°), 17 h (0°).
- (7) S + 0.1 c.c. D' + 0.2 c.c. rabbit's serum. } No A or L in (6) or (8). Slight
- (8) S + 0.1 c.c. heated D' + 0.2 c.c. rabbit's serum. } L but no A in (7). Fair L in
- (9) S + 0.1 c.c. NaCl solution + 0.2 c.c. D'. } in (9), but distinctly less than in (5). Fair A in (9).

D, whether heated or not, gives, in general, much better A than D'.

FURTHER PROOF OF ION ACTION IN PHYSIOLOGIC PROCESSES.

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THE purpose of this paper is to present additional evidence in support of the statement made by us¹ in a previous paper, that the results obtained from the action of electrolytes upon the decomposition of hydrogen dioxide by platinum black, and by a watery extract of pancreas, could be explained, in general, by the assumption that the cations or positive ions have a depressing or retarding action and the anions or negative ions have a stimulating action. Kastle and Loevenhart² in a recent article in *Science* took exception to the use of the term "ion action" by physiologists and pharmacologists in general, and by the writers of this article in particular. Our statement, that the negative ion stimulates, and the positive ion inhibits catalytic action does not imply that the negative or positive charge is necessarily the active factor. We merely gave the facts and offered no explanation as to how the stimulation or depression was produced. The influence of ions may be due solely to the charge, and to the physical properties of the ion, as given by Dr. A. P. Mathews³ in a recent paper. Again, it may be explained, according to Kastle and Loevenhart, by the formation of insoluble films. It seems to be just for us to remark that Kastle and Loevenhart apparently see no explanation for the stimulating action which some electrolytes exert upon the decomposition of hydrogen dioxide by finely divided metals. Again, will this theory of an insoluble film hold when soluble ferments are substituted for the finely divided metals?

The theory of the dissociation of a compound into ions has been so thoroughly substantiated by such numerous and brilliant examples

¹ NEILSON and BROWN: This journal, 1904, x, p. 335.

² KASTLE and LOEVENHART: *Science*, 1904, xix, p. 630.

³ A. P. MATHEWS: This journal, 1904, xi, p. 455.

that it need no longer be considered a theory. Jones¹ says, "Since the theory was proposed, it has been tested both theoretically and experimentally from many sides; with the result that, when all the evidence available is taken into account, the theory of electrolytic dissociation seems to be as well established as many of our so-called laws of nature."

Dry metallic sodium and dry sulphuric acid do not react with each other. Dry hydrochloric acid and dry fumes of ammonia may be mixed without chemical change. Hydrochloric acid in toluol possesses none of its acid properties, *i. e.*, it is not dissociated.

Osmotic pressure depends on the number of particles in solution. Ionization explains why a solution of sodium chloride has a higher osmotic pressure than a sugar solution of the same concentration.

This also explains the too high molecular weight of salts when determined by the freezing point methods. It also places the question of chemical equilibrium and the facts of precipitation on a definite mathematical basis. Substances, as urea, sugar, etc., known as non-electrolytes are relatively inactive chemically, while the electrolytes, or acids, bases, and salts, are active chemically.

In the application of the Arrhenius theory of electrolytic dissociation to the explanation of physiological processes, the names of Loeb in animal physiology, and Kahlenberg and True in plant physiology, stand out as pioneers. Loeb² found that the gastrocnemius muscle of the frog contracts rhythmically only in solutions of electrolytes, the non-electrolytes being ineffective. Kahlenberg and True³ found that solutions of acids, bases, and salts, completely dissociated, owe their toxicity on the bean *Lupinus albus* L. to the specific ions in solution. Lingle's⁴ work with electrolytes and non-electrolytes on turtle's heart further substantiates the view that the phenomenon of a contracting heart strip is dependent on specific ions.

A. P. Mathews⁵ shows that the physiological effect of electrolytes is due to ions, and that this action is due to the electrical and physical properties of these ions.

Howell's work on the heart, Ringer's work, and the work of a host of others can be satisfactorily explained by the action of ions in so far as their action on protoplasm is known.

¹ JONES: The Modern Theory of Solutions, p. 9.

² LOEB: Archiv für die gesammte Physiologie, 1899, lxi, p. 99.

³ KAHLENBERG and TRUE: Botanical gazette, 1896, xxii, p. 81.

⁴ LINGLE: This journal, 1900, ii, p. 205.

⁵ MATHEWS: *Loc. cit.*

The inversion of sugars by acids may, perhaps, be considered as truly a physiological process as when it is brought about by animal or vegetable diastases. It has long been known that the power of an acid to invert a sugar is in direct ratio to the number of hydrogen ions present. The strongly dissociated hydrochloric acid splits in a given time an amount which may be designated as 100 per cent, while the weakly dissociated acetic acid splits in the same length of time only a fraction of 1 per cent. The importance of hydrochloric acid in the stomach in a concentration where dissociation is very great is an example of ion-function well known to all. Neilson and Brown,¹ Kastle and Loevenhart,² Cole,³ McGuigan,⁴ and others have demonstrated clearly that enzymic activity in the presence of a salt solution is dependent on the ions in the solution.

The experimental data of this paper will be presented in three divisions, as follows: Part I, the influence of the non-electrolytes upon the decomposition of hydrogen dioxide by platinum black and a watery extract of kidney; Part II, the influence of salts with less degree of ionization than normal, upon the decomposition of hydrogen dioxide by platinum black; Part III, the effects of a compound with one ion suppressed, upon the decomposition of peroxide of hydrogen by platinum black, and a watery extract of kidney.

METHODS.

The platinum black was mixed with distilled water and measured out while the mixture was being constantly stirred. The constancy of the controls showed that equal amounts of the metal were measured out each time. A watery extract of kidney was used instead of the watery extract of pancreas which was used in our former experiments. The kidney extract is more stable than the pancreas extract, in which autolytic processes are rapid. The kidney extract is also more active than the pancreas extract, and at the strength necessary to produce the desired rate of evolution of gas it has much less albuminous material than the pancreas extract producing the same evolution of oxygen, and therefore it is much more accurately and easily measured by a pipette. Fresh beef kidney was used, and the extract was made

¹ NEILSON and BROWN: *Loc. cit.*

² KASTLE and LOEVENHART: *American chemical journal*, 1903, xxix, p. 563.

³ COLE: *Journal of physiology*, 1903, xxx, p. 202.

⁴ MCGUIGAN: *This journal*, 1904, x, p. 444.

by mincing and grinding the kidney in a mortar, adding water, and filtering through filter paper. Two large-mouthed bottles of 200 c.c. capacity were fitted with double-holed rubber stoppers. From one hole of each stopper, by means of rubber and glass tubing, a connection was made with a eudiometer tube of 75 c.c. capacity. After the solution to be tested and the kidney extract had been put into the bottles, the stoppers were adjusted, and through the second holes in the stoppers, which were about a quarter of an inch in diameter, the hydrogen dioxide was introduced simultaneously by means of two pipettes. Immediately and at the same time the two holes were closed by glass rods. By this method of procedure and the use of the

TABLE I.
PLATINUM BLACK.

SUBSTANCES.	CONCENTRATIONS.							
	m 1		m 5		m 64		m 512	
	Cubic centimetres of oxygen given off in							
	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Sugar	11	20	13	27	13	27	13	27
Urea	2	6	10	16	13	27	13	27
Glycerin	13	27	13	27	13	28	12	27
Alcohol (ethyl)	12	27	13	26	13	27	12	27
Water	13	27

watery extract of kidney or the platinum black, prepared as given above, the control experiments did not vary more than 1 c.c. of oxygen without some apparent cause. The amount of oxygen given off was recorded at the end of one and two minutes. To insure the free liberation of the gas, the bottles were shaken. In order to have a uniform shaking, the bottles were fitted in holes in a block of wood which was shaken by hand. The shaking might have been done by a mechanical device, but the results were so constant that variations in shaking can be ignored. Unless otherwise stated, the amount of water or the solution to be tested was 25 c.c.; the amount of kidney extract, 5 c.c.; the amount of hydrogen dioxide, 10 c.c. The kidney

extract and the peroxide were kept in ice-water, and the solution was kept at room-temperature. The dioxide of hydrogen used was that made by the Mallinckrodt company of St. Louis.

I. THE ACTION OF NON-ELECTROLYTES.

Solutions of non-electrolytes, as urea, glycerin, sugar, etc., in concentrations isotonic with the blood, do not stimulate nerves. If the solution has a concentration giving an osmotic pressure of fourteen atmospheres or more, the nerve is stimulated if placed in it. This is due to a purely physical process, — *i. e.*, the extraction of water from the nerve. By the use of non-electrolytes we have a way of deter-

TABLE II.
KIDNEY EXTRACT.

SUBSTANCES.	CONCENTRATIONS.											
	Cubic centimetres of oxygen evolved in											
	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Sugar	3	8	5	12	8	14	8	15	8	15
Urea	1	2	4	7	5	10	6	11	8	15	8	15
Glycerin . .	6	12	8	15	8	15	8	15	8	15	8	15
Alcohol (ethyl)	4	7	8	14	8	15	8	15	8	15	8	15
Water. . .	8	15

mining whether molecules as such have any action upon the decomposition of the peroxide of hydrogen by platinum black and a watery extract of kidney. Weiss¹ and Arnheim² found that a solution of gum-arabic accelerated pepsin digestion; but Mugdan³ showed pepsin digestion to be delayed by gum-arabic. Meisenheimer⁴ found zymase action delayed by glycerin solutions. Braeuning,⁵ who tried

¹ WEISS: *Zeitschrift für physiologische Chemie*, 1897, xl, p. 488.

² ARNHEIM: *Zeitschrift für physiologische Chemie*, 1897, xl, p. 238.

³ MUGDAN: *Berliner klinischen Wochenschrift*, 1891, p. 788.

⁴ MEISENHEIMER: *Zeitschrift für physiologische Chemie*, 1897, xxx, p. 520.

⁵ BRAEUNING: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 80.

many non-electrolytes on different kinds of ferment action, sums up his work by saying, the stronger the solution of the non-electrolyte, the more the ferment action will be delayed. Our results are similar, as is shown in Tables I and II. When the concentration is 5 mol or more, there is considerable inhibition; but in the 1 mol and weaker there is little or no effect. Urea has a greater inhibitory action than the other substances in the same concentration. This may be explained by the fact that ionization does occur to a limited extent in urea. This was shown by the ability of the solution of urea which we used to conduct the current better than could the distilled water from which it was made.

TABLE III.

<i>z.</i>	Water. <i>u_γ</i>	Methyl-alcohol. <i>u_γ</i>	Methyl-alcohol $\frac{1}{2}$, water $\frac{1}{2}$. <i>u_γ</i>
16	123.1	..	59.82
32	127.5	69.02	62.46
64	130.5	76.70	65.36
128	132.9	83.60	67.11
256	136.4	88.96	69.26
512	140.2	93.26	70.53
1024	143.4	97.25	..

II. SALTS WITH LESSENERED IONIZATION.

The power of dissociating the molecules of a salt into its ions differs in various solvents. Water is the strongest ionizer known, with the possible exception of hydrogen dioxide. The alcohols are considerably weaker in their ionizing power. Wakeman¹ showed that the addition of water to a solution of an organic acid in ethyl-alcohol increased proportionately the conductivity of the solution. Zelinsky and Krapivin² found, however, that substances dissolved in methyl-alcohol presented an exception. It was shown by these workers that certain salts had a higher conductivity in pure methyl-

¹ WAKEMAN: *Zeitschrift für physikalische Chemie*, 1893, xi, p. 49.

² ZELINSKY and KRAPIVIN: *Zeitschrift für physikalische Chemie*, 1896, xxi, p. 189.

alcohol than in a 50 per cent solution. This is shown by Table III taken from the work of Zelinsky and Krapivin, in which v equals the number of litres containing a gram molecule, and uv equals the molecular conductivity.

It occurred to us that, if the stimulating or depressing power of a salt upon the hydrolysis of hydrogen dioxide by platinum black, depended at all upon the ionic condition of the salt, we should be able to obtain a difference in the amount of oxygen given off when the salt was dissolved in water alone, and when it was dissolved in equal parts of water and methyl-alcohol. Of course the retarding influence of the alcohol was necessarily taken into consideration. As

TABLE IV.
PLATINUM BLACK.

SUBSTANCES.	Cubic centimetres of oxygen liberated in	
	1 min.	2 min.
25 c.c. water	60	88
25 c.c. 50% methyl-alcohol	24	35
25 c.c. $\frac{11}{8}$ calcium chloride in water	24	40
25 c.c. $\frac{11}{8}$ calcium chloride in 50% methyl-alcohol . .	11	19
25 c.c. water	60	88
25 c.c. 50% methyl-alcohol	24	35
25 c.c. $\frac{11}{400}$ mercuric chloride in water	5	10
25 c.c. $\frac{11}{400}$ mercuric chloride in 50% methyl-alcohol .	4	7

the methyl-alcohol has a precipitating action upon proteids, the experiment was not performed with the kidney extract. The results will be seen in Table IV.

It will be observed from Table IV that the amount of oxygen given off in one minute in the methyl-alcohol is 36 c.c. less than that in pure water. This represents the inhibitory effects of the alcohol. In the calcium chloride there is also 36 c.c. less than in the water. The sum of these, 72 c.c., then represents the inhibitory effects of the mixture of calcium chloride and methyl-alcohol, if each one exerts, when mixed, the full power that it possesses when separate. But these

two substances mixed together allowed 11 c.c. to come off. Therefore their inhibitory power when mixed is the remainder of sixty minus eleven, which is forty-nine. Then the lessened inhibitory power is the remainder of seventy-two minus forty-nine, which is twenty-three. The only explanation that we can see for this is the decreased dissociation of the calcium chloride when dissolved in equal parts of methyl-alcohol and water. By making a calculation of the amounts at the end of the second minute, it will be found that the lessened inhibition corresponding to the lessened dissociation is 32 c.c. of oxygen. When mercuric chloride is used instead of the calcium chloride, the result is still more striking. By calculating as before, it will be found that the lessened inhibition of the mercuric chloride for one and two minutes is respectively 35 c.c. and 50 c.c. of oxygen. This was also tried upon the stimulating salts and was found to hold.

III. THE EFFECT OF LESSENING THE CONCENTRATION OF EITHER THE POSITIVE OR NEGATIVE ION.

In qualitative and quantitative chemistry, advantage is taken of the fact that the addition of a common ion to a substance in solution lessens the dissociation of that substance. Based upon the equilibrium of electrolytes, the fact is well known that a weak base, as ammonia hydroxide, is made still weaker by the addition of an ammonium salt or another hydroxide. Likewise a weak acid is made still weaker by the addition of one of its salts. For example, acetic acid by the addition of sodium acetate has a much weaker acid effect. The addition of the common acetate ion depresses the dissociation of the acetic acid, and thus lessens the number of hydrogen ions. This is shown by the following formula:

Equilibrium equation = $\frac{H \times CH_3COO}{CH_3COOH} = k$, or the constant of acetic acid dissociation = $\frac{1}{50000}$. During the first interval of time after adding sodium acetate the formula becomes

$\frac{H \times CH_3COO + x \text{ acetate ions}}{CH_3COOH} > k$ for acetic acid. When constant equilibrium is established we have $\frac{(H-y)(CH_3COO+x-y)}{CH_3COOH+y} = k$.

That is, acetate ions plus hydrogen ions forms molecular acetic acid, which decreases the product of ion concentration in the numerator, and increases the denominator, thereby decreasing the dis-

sociation and the number of H ions. In the application of this fact to physiological problems not much experimental work has been done. Paul and Kronig¹ found that the addition of sodium chloride to mercuric chloride caused the toxicity or the germicidal action of the latter to be lessened. They found that anthrax spores lived longer in a mixture of the sodium and mercuric chlorides than in the

TABLE V.

SUBSTANCES.	Cubic centimetres of oxygen liberated in	
	1 min.	2 min.
25 c.c. water	46.0	72.0
20 c.c. water + 5 c.c. $\frac{1}{200}$ mercuric chloride	15.0	26.0
20 c.c. $\frac{1}{8}$ calcium chloride + 5 c.c. water	29.0	46.0
20 c.c. $\frac{1}{8}$ calcium chloride + 5 c.c. $\frac{1}{200}$ mercuric chloride	5.0	9.0
25 c.c. water	46.0	72.0
23 c.c. water + 2 c.c. $\frac{1}{200}$ mercuric chloride	27.0	44.0
23 c.c. $\frac{1}{8}$ calcium chloride + 2 c.c. water	26.0	41.0
23 c.c. $\frac{1}{8}$ calcium chloride + 2 c.c. $\frac{1}{200}$ mercuric chloride	12.5	18.5
25 c.c. water	46.0	72.0
24 c.c. water + 1 c.c. $\frac{1}{200}$ mercuric chloride	37.0	57.0
24 c.c. $\frac{1}{8}$ calcium chloride + 1 c.c. water	26.0	42.0
24 c.c. $\frac{1}{8}$ calcium chloride + 1 c.c. $\frac{1}{200}$ mercuric chloride	21.0	32.0

mercuric chloride alone. Their explanation for this was, that the common chlorine ion decreases the dissociation of the mercuric chloride, and therefore the number of mercuric ions is lessened. These workers found also that the germicidal action of mercuric chloride, bromide, and cyanide is dependent in the main on the concentration of the mercuric ions, *i. e.*, upon the degree of dissociation

¹ PAUL and KRONIG: *Zeitschrift für Hygiene und Infektions-Krankheiten*, 1897, xxv; cited from COHEN's Physical Chemistry.

of the salts. Similar results were also obtained with gold, silver, and copper salts.

In testing the concentration of the positive ions upon the rate of catalysis of hydrogen dioxide, we used mercuric chloride as the solution to be tested, and to this was added sodium chloride or calcium chloride. The addition of the common chlorine ion decreases the dissociation of the mercuric chloride by increasing the amount of

TABLE VI.
KIDNEY EXTRACT.

SUBSTANCES.	Cubic centimetres of oxygen liberated in	
	1 min.	2 min.
25 c.c. water	15.0	30.0
20 c.c. water + 5 c.c. $\frac{n}{300}$ calcium acetate	26.0	45.0
20 c.c. $\frac{n}{8}$ calcium chloride + 5 c.c. water	4.0	7.0
20 c.c. $\frac{n}{8}$ calcium chloride + 5 c.c. $\frac{n}{300}$ calcium acetate	10.5	19.0
25 c.c. water	15.0	30.0
23 c.c. water + 2 c.c. $\frac{n}{300}$ calcium acetate	19.0	36.5
23 c.c. $\frac{n}{8}$ calcium chloride + 2 c.c. water	3.0	6.0
23 c.c. $\frac{n}{8}$ calcium chloride + 2 c.c. $\frac{n}{300}$ calcium acetate	5.5	10.0
25 c.c. water	15.0	30.0
24 c.c. water + 1 c.c. $\frac{n}{300}$ calcium acetate	17.0	33.0
24 c.c. $\frac{n}{8}$ calcium chloride + 1 c.c. water	3.5	6.0
24 c.c. $\frac{n}{8}$ calcium chloride + 1 c.c. $\frac{n}{300}$ calcium acetate	3.5	6.0

molecular mercuric chloride. If now the mercury ion has a depressing action on the rate of the decomposition of hydrogen peroxide by platinum black or a watery extract of pancreas, the addition of calcium chloride ought to lessen this depressing action. Such was found to be the case. By consulting the first part of Table V where 5 c.c. of $\frac{n}{200}$ mercuric chloride were used, it will be noticed that 72 c.c. of oxygen were liberated at the end of the second minute in water. Where the mercuric chloride was used, 26 c.c. were given off.

The depression of the mercury then was 46 c.c. of oxygen. Where the calcium was used alone, 46 c.c. of oxygen were freed. The depressing effect of the calcium was 26 c.c. of oxygen. When these two salts are mixed, if each exerts its full inhibitory power, there should be an inhibition of the normal catalysis of 72 c.c. of oxygen, or there should be nothing given off. This, however, is found not to be the case. 9 c.c. of oxygen were liberated at the end of the second

TABLE VII.

SUBSTANCES.	Cubic centimetres of oxygen liberated in	
	1 min.	2 min.
25 c.c. water	15.0	30.0
20 c.c. water + 5 c.c. $\frac{N}{500}$ sodium acetate	26.0	47.0
20 c.c. $\frac{N}{8}$ sodium chloride + 5 c.c. water	5.0	9.0
20 c.c. $\frac{N}{8}$ sodium chloride + 5 c.c. $\frac{N}{500}$ sodium acetate .	12.5	22.5
25 c.c. water	15.0	30.0
23 c.c. water + 2 c.c. $\frac{N}{500}$ sodium acetate	19.0	37.0
23 c.c. $\frac{N}{8}$ sodium chloride + 2 c.c. water	3.0	6.0
23 c.c. $\frac{N}{8}$ sodium chloride + 2 c.c. $\frac{N}{500}$ sodium acetate .	6.0	10.5
25 c.c. water	15.0	30.0
24 c.c. water + 1 c.c. $\frac{N}{500}$ sodium acetate	18.0	35.5
24 c.c. $\frac{N}{8}$ sodium chloride + 1 c.c. water	3.0	5.5
24 c.c. $\frac{N}{8}$ sodium chloride + 1 c.c. $\frac{N}{500}$ sodium acetate .	5.0	8.5

minute in the mixture. The same results will be noticed at the end of the first minute, and also where the smaller amounts of mercuric chloride are used. An objection to this might be made, — namely, that the chlorine ions of the mercuric chloride lessened the dissociation of the calcium chloride. This, however, is not the case. Calcium chloride is a strongly dissociated salt, while the mercuric chloride is a weakly dissociated salt. In case the calcium chloride is lessened in degree of dissociation a small amount, the results would not be

vitiated, as this would lessen the depressing influence of the calcium ion, and would also furnish proof of the point in hand.

To show the effect of lessening the number of the anions or negative ions, a similar method was used. For the solutions to be tested we used sodium and calcium acetates, and added in the one case sodium chloride and in the other calcium chloride. The addition of the common ion, sodium, in the one case and calcium in the other, will lessen the dissociation of the sodium or calcium acetate, and thereby will decrease the number of acetate ions, correspondingly the stimulation should be less. The results are seen in Tables VI and VII. By subtracting the amount of oxygen given off in water at the end of the second minute, from that given off in the water and calcium acetate, the stimulating action of the acetate will be found. This is 16 c.c. of oxygen. By subtracting the amount of oxygen given off at the end of the second minute, in the water and calcium chloride, from that given off in the calcium chloride and calcium acetate, the stimulating action of the acetate in the presence of calcium chloride will be found. This is 12 c.c. of oxygen. The stimulation of the calcium acetate is seen to be 4 c.c. of oxygen less, when in presence of the calcium chloride, than where the calcium acetate was used in water alone. Similar results are obtained in the other concentrations, but it is especially striking where 1 c.c. of the calcium acetate was used. In this amount the calcium acetate in presence of the calcium chloride has no stimulating effect, while in presence of water it stimulates 3 c.c. of oxygen. Sodium acetate is affected by sodium chloride the same as the calcium acetate is by the calcium chloride. The explanation for this is the same as before given, — namely, the strongly dissociated sodium chloride or calcium chloride, when added to the less strongly dissociated sodium acetate or calcium acetate decreases the dissociation of the latter and thereby decreases the number of stimulating negative ions. The same results were obtained with platinum black as were obtained with kidney extract.

CONCLUSIONS.

1. The non-electrolytes have no effect except in solutions of 1 mol concentration, or more, upon the decomposition of hydrogen dioxide by platinum black, or by a watery extract of kidney; in concentrations stronger than 1 mol, there is an inhibitory effect which increases with the increasing concentration.

2. A salt in dilute concentration exerts either a depressing or stimulating effect upon the decomposition of hydrogen peroxide by platinum black, or a watery extract of kidney, by virtue of its ionic condition.

3. The stimulating effect of a salt upon the splitting of peroxide of hydrogen by platinum black, or a watery extract of kidney, depends upon the negative ion, while the retarding effect depends upon the positive ion.

Our thanks are due Professor Lyon for valuable criticisms and suggestions.

THE PASSAGE OF DIFFERENT FOOD-STUFFS FROM THE STOMACH AND THROUGH THE SMALL INTESTINE.¹

By W. B. CANNON.

[From the Laboratory of Physiology in the Harvard Medical School.]

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IN 1901, during the course of observations on the movements of the intestines, I noted that salmon began to leave the stomach later than bread and milk, and that it was slower in reaching the large intestine; in the report of the research I called attention to this interesting difference.² It seemed that a careful study of the manner in

¹ A partial report of this research was presented at the meeting of the American Physiological Society in December, 1903, and was published in the Proceedings, This journal, 1904, x, p. xvii.

² CANNON: This journal, 1902, vi, p. 263.

which the different food-stuffs are mechanically treated by the alimentary canal might suggest the mechanisms by which the movements are controlled.

The purpose of the following investigation, therefore, was primarily to study the mechanical treatment of the various food-stuffs in the stomach and small intestine. This study was to include the rate of gastric peristalsis and of rhythmic segmentation in the small intestine, and such important matters as the time-interval between eating and the first passage of food from the stomach, the rate at which the stomach empties, and the time required for traversing the small intestine. It was also desirable to know how these processes might be affected by altering the conditions, as, for example, by mixing different kinds of food-stuffs, and by changing their amount. In order to serve for a study of this nature the method should be as simple and exact as possible, and it should not interfere with the process of digestion or with the course of the food through the digestive canal.

THE METHOD.

Among the first essentials for simplicity of method in a study of the agencies controlling the mechanical activities of the stomach and intestines is the employment of food-stuffs as purely proteid, fat, or carbohydrate as possible. Such foods were used in this investigation. Boiled beef free from fat, boiled haddock, and the white meat of fowl are examples of proteids which were fed; beef suet, mutton and pork fat are representatives of the fats; starch paste, boiled rice, and boiled potatoes, of the carbohydrates. The foods were invariably given in uniform amount, 25 c.c. They were always finely shredded or pressed in a mortar, and were moistened with sufficient water to produce, as nearly as could be judged by the eye and by manipulation, the uniform consistency of thick mush. Before the food was fed to the animals, it was mixed with 5 grams of subnitrate of bismuth. A comparison of subnitrate of bismuth with other insoluble heavy salts has shown that it has no peculiar effects on the movements of the alimentary canal; and also clinical studies by Schüle¹ have proved that the addition of subnitrate of bismuth to food does not interfere with normal gastric motility.

¹ SCHÜLE: Zeitschrift für klinische Medicin, 1896, xxix, p. 67.

In all cases full-grown cats, deprived of food for the twenty-four or thirty hours previous to the experiment, served for the observations. The animals were either permitted to eat voluntarily from a dish, or were placed on the holder and were fed from a spoon, usually with little or no difficulty. The animals thus fed with food mixed with bismuth subnitrate were exposed to the X-rays and, without disturbing the processes of digestion, the movements of the food in the stomach and small intestine were observed by means of the shadows cast on a fluorescent screen. That the results secured were not due to individual peculiarities of the cats was proved by using the same cat repeatedly with different food-stuffs, and finding the results characteristic of the food, and not peculiar to the cat. Animals once used were not used again within three days.

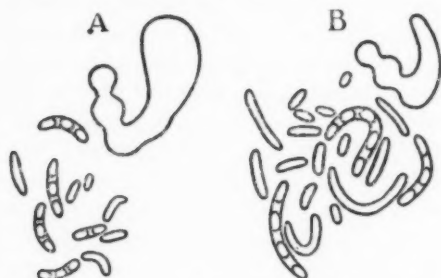


FIGURE 1. — Tracings of the shadows of the contents of the stomach and intestine, made two hours after feeding boiled lean beef (*A*) and boiled rice (*B*). The aggregate length of the shadows of the intestinal contents in *A* was 20 cms., in *B*, 43 cms. The small divisions of the food in some of the loops represent the process of rhythmic segmentation. One-third original size.

The observations were recorded at regular intervals, — one-half hour, one hour, and then every hour for seven hours after the feeding. The records consisted of outlines of the shadows traced on transparent tissue paper laid over the fluorescent surface. If in any case there was doubt that all the shadows had been recorded, an electric light was flashed momentarily on the tracing before it was removed from the screen, and thus the outlines drawn on the paper were compared with the shadows cast by the intestinal contents, and the outlines verified.

Inasmuch as the diameter of the small intestine varies only slightly (see Fig. 1), the area of cross-section of the contents may be disregarded, and the aggregate *length* of the shadows of the contents

of the small intestine may be taken to indicate the amount of food present. By comparing the aggregate lengths of these shadows it is possible to determine the relative amounts of food in the intestine at different times after feeding, as well as the relative amounts of different foods in the intestine in different cases at the same time after feeding. For example, in the original tracings, represented in Fig. 1, the amount of proteid in the intestine two hours after feeding, indicated by the aggregate length of the masses, was 20 centimetres; and the amount of carbohydrate, similarly indicated, was 43 centimetres. By this method the observer, without interrupting or interfering with the course of digestion, can know when food first leaves the stomach, the rate at which different food-stuffs are discharged into the intestine, the interval till food first appears in the large intestine, and the mechanical treatment which the food receives as it passes through the canal.

The method has obvious defects. The loops of intestine are not always parallel to the screen, and the loops not parallel may not always make the same angles with the screen; the shadows cast by contents of the loops would therefore be variously foreshortened. In extenuation of this defect, it may be said that the animals were stretched on their backs, and that the ventral abdominal wall was flattened against the back, both by the stretching and by the pressure of the fluorescent screen; the loops must therefore have been nearly parallel to the screen, except at possible short dorso-ventral turns from one loop to another. That the foreshortening of the shadows in the loops and turns was not a serious source of error was repeatedly proved by tracings made before and after a rearrangement of the loops by massage of the abdomen; the tracings showed only slight variations in the aggregate length of the shadows. Another possible defect of the method arises from the chance of such overlapping of the loops that two masses of food or parts of two masses may cast a single shadow. Care was invariably taken to obviate this error by pressing apart with the fingers loops lying close together. A further criticism of the method suggests itself, — that the bismuth subnitrate and the food may separate, and that the shadows may then be misleading. Several animals were fed the three different kinds of food, and were killed at intervals from two to six hours after the feeding. The intestinal mucosa was remarkably free from any perceptible separate deposits of the heavy powder, and the well-limited masses of material scattered at intervals along the gut were mixtures of sub-

nitrate of bismuth and the food. Naturally, with the digestion of a part of the food and with the constant interchange of fluids between the intestinal mucosa and the food-remnant in its movement onward, the relation of the bismuth subnitrate to the remnant must vary; but examination proved that the remnant does not become fluid to a degree which prevents it from being a vehicle of transmission for the bismuth salt, nor, on the other hand, does the percentage of bismuth fall until it no longer indicates the presence of alimentary material. It is clear that the changes in the relation of the bismuth subnitrate to the food, on account of absorption of the food or secretion of digestive fluids, are much less in the early stages of intestinal digestion, when no absorption and but little digestive alteration have taken place, than they are later. The application of the method to the determination of the rate of discharge through the pylorus is therefore justified only in the first two or three hours of digestion before much absorption has occurred. Other minor faults of the method are to be found in the variations in the thickness of the food-masses at different times, and in the individual rates of absorption for the different foods. These defects, however, must be regarded, especially in the early stages of intestinal digestion, as relatively slight, compared with the great and characteristic differences in the amounts of food present in the intestine when carbohydrates, fats, and proteids are separately fed.

In securing data in this research about 1200 observations were made on 150 cases. A few of these cases have not been reported because they were evidently pathological, — the animals were afflicted with coryza and conjunctivitis, and almost no food left the stomach for at least seven hours; other cases have not been reported, because they were preliminary and did not afford data for the regular times selected for the observations. Furthermore, the desirability of a uniform number of cases, for comparing different conditions, required, at the time the results were collated, either the exclusion of the cases in excess of the smallest number for any condition, or many more observations to bring the smaller up to the larger numbers. The degree of uniformity of the results justified the former course, and many cases were thus excluded. The 728 observations on 91 cases reported in the following sections are thoroughly representative.

THE TREATMENT OF THE DIFFERENT FOOD-STUFFS
BY THE STOMACH.

The rate of gastric peristalsis. — In observations made on the stomach in 1897¹ I noted that the peristalsis of the stomach was to be seen whenever an animal was examined during gastric digestion, and I inferred that peristaltic waves are running continuously throughout the entire digestive period. All observations made since have supported that inference, — so long as food remains in the stomach the waves, ever-recurring, sweep slowly, one after another, from the middle of the stomach to the pylorus. The rate of peristalsis noted when bread and milk were fed was stated as 6 waves per minute; a new contraction would thus appear every ten seconds. A considerably slower rate of peristalsis (4 waves per minute), noticed after feeding a fat, suggested that there might be characteristic rates for the different food-stuffs. Observations at different intervals after feeding were therefore made on various animals which had been fed various kinds of foods, and the following results secured:

	Average rate per minute.	Rate most frequently observed.	Extreme variations in rate.
	WAVES	WAVES	WAVES
Fats (23 observations)	5.0	5.2	4.0-6.0
Proteids (16 observations) . .	5.2	5.0-5.4	4.8-5.8
Carbohydrates (13 observations)	5.5	5.8	5.0-6.0

The average rate of peristalsis with the separate food-stuffs differs concomitantly with the rate most frequently observed, but the difference is relatively so slight, and the variation with any given food so great, as to make it improbable that each food-stuff has a characteristic rate.

The variation during a period of digestion suggested in some cases a slowing of peristalsis as time passed. The following examples, however, showing the number of waves per minute at different times after feeding different foods, prove that no general statement can safely be ventured.

¹ CANNON: This journal, 1898, 1, p. 367.

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Bacon fat	4.6	5.4	5.0	5.2	4.6	5.4	4.8	5.0
Boiled haddock	5.4	5.4	5.6	..	5.0	5.0	5.0	4.8
Boiled rice	5.8	5.8	5.4	..	5.2

It will be shown later that, when given in fairly pure forms, carbohydrates leave the stomach first, proteids next, and fats slowest of all. The figures presented in this section show that the average rates of peristalsis follow this same order,—carbohydrates most rapid, proteids next, and fats slowest. The differences, however, are so slight that the slower rate does not compensate for the greater duration of stay in the stomach. When equal amounts of carbohydrate, proteid, or fat food are given, a much larger number of peristaltic waves, therefore a much greater expenditure of energy in muscular contraction, is required by the proteids and fats than by the carbohydrates, before the stomach is emptied.

The rate of discharge from the stomach.—Tables of the “digestibility” of foods, as indicated by the time during which the various foods remain in the stomach, have long been published. Beaumont¹ made such a table from his observations on Alexis St. Martin, and more recently Leube,² and Penzoldt³ and his pupils have studied, by means of the stomach tube, the duration of the gastric digestion of various foods, and tabulated their findings. The use of these figures to judge the rate at which the stomach is emptied is open to criticism. First, the observations were made either on a pathological subject or on one whose digestion was interrupted by the introduction of a stomach tube. The results, furthermore, express merely the time when the stomach was found empty; they give no hint as to the moment when food first passed the pylorus, or as to the amounts, large or small, which entered the intestine at any stage during digestion. Also, if comparisons are to be made, it is obviously necessary to note the amount of food given. Beaumont’s records indicate frequent inattention to this factor, and Leube’s observations

¹ BEAUMONT: *The Physiology of Digestion*, second edition, Burlington, 1847, p. 292.

² LEUBE: *Zeitschrift für klinische Medicin*, 1883, vi, p. 189.

³ PENZOLDT: *Deutsches Archiv für klinische Medicin*, 1893, li, p. 545.

have the same defect. Although Penzoldt recorded the amounts of food given, he did not give systematically the same amounts, so that the stomach was not always dealing with the same mechanical problem. And these investigators did not consider in their comparisons the consistency of the food, a factor which Moritz¹ has rightly noted as important. Moreover, the endeavor of these investigators was to learn the manner in which ordinary articles of diet were treated in the stomach,—the simplification of the conditions by the use of fairly pure food-stuffs they did not much regard.

The research here reported was an attempt to learn how different food-stuffs, other factors being as nearly as possible the same, would be acted on mechanically by the stomach and small intestine. It was the intention also to discover, if possible, the agents determining the difference in treatment. Fairly pure food-stuffs, therefore, were administered; they were given in uniform amount; they were, as nearly as could be judged by the eye and by manipulation, uniform

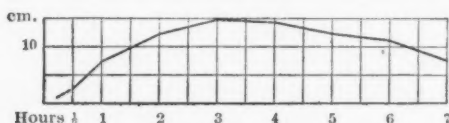


FIGURE 2. — Curve showing average aggregate length in centimetres of the masses of fat food present in the small intestine at regular intervals, for seven hours after feeding various fat foods. Sixteen cases.

in consistency. It is to be remembered that the results here stated are significant for the emptying of the stomach chiefly in the early stages of intestinal digestion, before much absorption has taken place.

1. *Rate of discharge of fats.* — In selecting the fats for this investigation, it was necessary to pay particular attention to the differences in the consistency of the fat at different temperatures. A fat of proper consistency at room-temperature might be much too fluid at body-temperature. Care was taken to choose fats or fatty tissues which, when mixed with subnitrate of bismuth, presented at body-temperature about the same degree of viscosity as the carbohydrate and proteid preparations.

The rate at which fats leave the stomach may be seen in the following average figures, representing in centimetres the aggregate

¹ MORITZ: *Zeitschrift für Biologie*, 1901, xlii, p. 565.

length of the food-masses in the small intestine, at regular times after the various fats were fed :¹

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Pork fat, 4 cases	6.7	11.6	12.2	18.0	16.5	13.7	14.1	9.0
Mutton tallow, 4 cases . .	0.6	3.7	6.2	9.5	8.1	9.4	9.0	7.5
Beef suet, 4 cases	1.6	9.0	16.2	14.5	10.7	6.6	5.4	6.7
Mutton fat, 4 cases	2.0	5.4	14.7	17.1	22.2	19.0	16.0	7.2
Average for fats, 16 cases .	2.7	7.4	12.4	14.8	14.4	12.2	11.1	7.6

The amount of variation observed in different animals when the same food (mutton tallow) was given, is illustrated in the following figures :

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine	2.5	4.5	4.5	11.0	4.0	4.5	7.0	5.0
	0.0	3.5	5.5	6.0	4.5	4.0	6.5	5.0
	0.0	0.0	7.0	9.0	10.0	13.0	13.5	11.0
	0.0	7.0	8.0	12.0	14.0	16.0	9.0	9.0
Averages	0.6	3.7	6.2	9.5	8.1	9.4	9.0	6.8

These tables and the accompanying curve of the fat-content of the small intestine (Fig. 2), plotted from the average figures of the sixteen cases of fat-feeding above reported, show that the emergence of fat from the stomach begins rather slowly, — in eight of the sixteen cases, indeed, it did not occur at all during the first half-hour of digestion, — and continues at such a slow rate that there is never any great accumulation of fat in the small intestine. Fats almost invariably are present in the stomach during the seven hours of observation; in one case an animal was killed six hours after receiving 25 c.c. of mutton fat, and about 11 c.c. of the food had not yet entered the intestine. The long, low curve, therefore, is the char-

¹ These fats were not cooked.

acteristic curve for fats. It indicates a discharge from the stomach at nearly the same rate at which the fat leaves the small intestine by absorption and by passage into the large intestine.

Zawilski,¹ in 1876, while studying the duration of the fat-stream through the thoracic duct, was impressed by the length of time necessary to complete the absorption of fat. His examination of three animals killed at different intervals after feeding fat mixed with other food, showed that about 100 of the 150 grams of fat were in the stomach after five hours of digestion, and even after twenty-one hours about 10 grams were still there. In the small intestine, on the other hand, the variation in amount was only slight: about 10 grams were found at five hours, and about 6 grams at twenty-one hours. Frank,² also, in investigating the absorption of fatty acids, noted in six animals that the fat stayed long in the stomach, and that in the small intestine a fairly uniform amount of fat was present at various times. Matthes and Marquadsen³ have confirmed Zawilski and Frank, but their observations, like the previous observations, were incidental to another investigation. The evidence that fats are retained long in the stomach is meagre, but the testimony of different observers is thus far harmonious. Recently, however, Strauss⁴ has denied that fats remain exceptionally long in the human stomach. But Strauss's methods are hardly comparable with the methods used by previous investigators. One hundred grams of butter and 1 litre of milk containing 9 per cent fat sufficed for the fat of the diet, but this was given with almost 600 grams of other food and 1700 c.c. of other drink. The observations were few, and on only one patient.

It is clear that the results tabulated above agree with and amplify the less complete evidence offered by Zawilski, Frank, and Matthes and Marquadsen. The long delay of fat in its passage through the alimentary canal is in the stomach. Fat passes from the stomach about as rapidly as the small intestine disposes of it; as a rule, therefore, the amount of fat in the small intestine is fairly constant in quantity and relatively slight in amount.

¹ ZAWILSKI: Arbeiten aus dem physiologischen Anstalt zu Leipzig, 1876, p. 156.

² FRANK: Archiv für Physiologie, 1892, p. 501.

³ MATTHES and MARQUADSEN: Verhandlungen des Congresses für innere Medicin, 1898, xvi, p. 364.

⁴ STRAUSS: Zeitschrift für diätetische und physikalische Therapie, 1899, iii, p. 279.

2. *Rate of discharge of carbohydrates.*—The following average figures, representing in centimetres the aggregate length of the food-masses in the small intestine at regular intervals after feeding, will indicate, particularly in the early stages of digestion, the rate at which various carbohydrate foods pass from the stomach.¹

Hours after feeding	$\frac{1}{2}$	1	2	3	4	5	6	7
Mashed potato, 4 cases . . .	9.4	30.9	43.0	25.2	21.2	13.7	9.2	4.1
Crackers and water, 4 cases .	11.0	22.0	35.4	39.5	40.0	27.5	22.1	12.1
Boiled rice, 4 cases	16.6	29.0	36.2	28.6	24.4	17.0	14.0	9.0
Starch pudding, 4 cases . . .	12.6	24.7	36.9	37.4	25.2	17.6	12.9	8.0
Av. for carbohydrates, 16 cases	12.4	26.6	37.7	32.7	27.7	19.0	14.0	8.3

The following figures illustrate the degree of variation observed when the same food (mashed potato²) was fed to different animals:

Hours after feeding	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine	9.0	30.0	43.5	28.0	22.0	19.5	9.0	0.0
	10.0	39.0	53.5	28.5	26.0	18.0	15.0	6.0
	9.5	22.0	36.0	36.5	30.0	16.5	13.0	10.5
	9.0	32.5	39.0	8.0	7.0	1.0	0.0	0.0
Average	9.4	30.9	43.0	25.2	21.2	13.7	9.2	4.1

In the accompanying curve, Fig. 3, plotted from the average figures for carbohydrates, the content of the small intestine at regular intervals after feeding is represented graphically. In my first observations on the movements of the stomach, bread was used to feed the animals, and I noted that this food appeared in the duodenum

¹ Glucose also was tried, but proved by itself to be such a violent stimulus to the motor activities of the alimentary canal that its use was not continued.

² MARBAIX (*La Cellule*, 1898, xiv, p. 299) notes that potatoes leave the human stomach rapidly, and that the gastric juice cannot attack them to any extent, and he suggests that an important question lies here.

within ten or fifteen minutes after feeding.¹ The tables and the curve for carbohydrates show that this early emergence of the starchy food from the stomach is followed by an abundant discharge. In a half-hour the amount present has almost equalled the maximum for fats, and at the end of an hour that amount has more than doubled. The abrupt, high rise of the curve to a maximum at the end of two hours indicates the rapidity of the rate of discharge. And as the stomach is usually empty about three hours after feeding carbo-

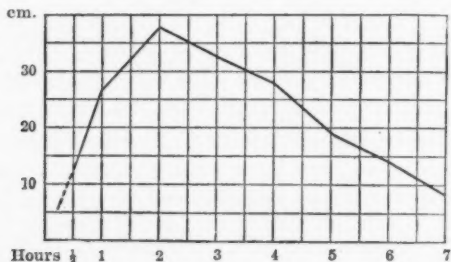


FIGURE 3.—Curve showing average aggregate length in centimetres of the masses of carbohydrate food present in the small intestine at regular intervals for seven hours after feeding various carbohydrate foods. Sixteen cases.

hydrates, the slow fall in the curve during the last four hours records the gradual departure of the food from the small intestine through the absorbing wall and into the colon.

Carbohydrates, therefore, begin to be discharged early from the stomach (ten or fifteen minutes after the food is swallowed), and the discharge is so rapid and abundant that the stomach is emptied in about three hours. The small intestine consequently receives a large amount in a relatively short time, and must be the resting-place for much of this food for a considerable period, for the passage of carbohydrates from the small intestine is usually slow and gradual.

3. *Rate of discharge of proteids.*—The rate at which various proteid foods pass from the stomach is indicated in the following average figures, which represent in centimetres the aggregate length of the food-masses in the small intestine at regular times after feeding :

¹ CANNON : This journal, 1898, i, p. 369.

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Fowl, 4 cases	1.8	6.6	13.1	18.7	20.6	23.5	18.0	15.4
Lean beef, boiled, 4 cases .	1.4	2.7	16.0	22.1	24.5	23.0	16.0	10.4
Fibrin, 4 cases	2.6	6.6	15.5	21.2	25.2	20.0	17.8	16.0
Haddock, boiled, 4 cases .	0.8	3.8	18.7	15.6	12.1	11.1	7.7	7.2
Av. for proteids, 16 cases .	1.6	4.9	15.8	19.3	20.6	19.3	14.7	12.2

A series of observations was made also with egg-albumin. The unchanged albumin, 25 c.c., mixed with 5 grams of subnitrate of bismuth and 3 grams of lycopodium spores (to offer a vehicle for the bismuth if the albumin should be absorbed) was first fed. The food passed from the stomach at the carbohydrate, not at the proteid rate. It seemed possible that the great rapidity of exit from the stomach might be due to the fluid state of the food, but when the same mixture of albumin, spores, and bismuth subnitrate was heated to a thick, jelly-like consistency, the rate of discharge was not diminished.¹

In the following table the first two cases were unchanged egg-albumin; the second two, egg-albumin coagulated by heat:

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine . . .	29.0	28.0	23.0	23.0	19.0	15.0	12.0	6.0
	20.0	33.0	22.0	24.0	20.5	13.0	7.0	6.0
	32.0	40.5	40.0	27.5	21.0	18.0	19.5	4.0
	17.5	34.5	46.5	29.0	16.5	13.5	5.0	2.0
Average	24.6	34.0	32.9	25.9	19.2	14.9	10.9	4.5

Comparison of these average figures with the averages of other proteids and of the carbohydrates, proves at once that egg-albumin is

¹ There is a marked discrepancy between these results and the statement made by ROUX and BALTHAZARD (*Archives de physiologie*, 1898, p. 91) that in the dog coagulated egg-albumin did not begin to leave the stomach for about three hours after feeding. Never have I seen so long a delay except in animals that were manifestly not in good health.

treated mechanically by the stomach much more like a carbohydrate than like other proteids. Gluten paste was also tried, and the averages of three observations were as follows:

Hours after feeding	$\frac{1}{2}$	1	2	3	4	5	6	7
	27.2	49.2	47.0	39.7	35.2	21.0	21.2	14.0

Here again the figures are like those from carbohydrates, not like those from proteids; but in this case the "gluten" was found to consist very largely of starch. No such explanation is at hand for the difference between the usual proteid averages and the averages for albumins, and any explanation of the differences between the mechanical treatment of proteids and of carbohydrates must take this exceptional treatment of albumin into consideration.

As an indication of the variation observed when the same food (boiled lean beef) is fed to different animals, the following figures are presented:

Hours after feeding	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine	0.0	2.5	9.5	14.0	18.0	21.5	12.0	9.0
	6.0	7.0	14.0	20.0	23.5	28.5	29.0	23.5
	0.0	4.0	11.0	19.0	19.0	21.0	21.0	21.0
	1.5	13.0	18.5	22.0	22.0	23.0	10.0	8.0
Average	1.8	6.6	13.1	18.7	20.6	23.5	18.0	15.4

Fig. 4 is a curve plotted from the average figures for the content of the small intestine after feeding the four representative proteids in the sixteen cases reported in the above table. Comparison of these average figures with the figures for the different proteids reveals a remarkable similarity during the first two hours of digestion. The striking feature of this part of the proteid curve (Fig. 4) is its very slow rise. In nine of the sixteen cases no food had left the stomach at the end of the first half-hour, and in eight cases the small intestine had not received at the end of an hour more than 4 centimetres of food. Some of the food usually remains in the stomach for about six hours; the fall in the curve, therefore, represents, as in the case of the fats, the resultant of the outflow from the stomach and of the departure

of the food from the small intestine, either by absorption or by moving onward into the colon. The difference between the initial discharge of carbohydrates and of proteids makes a further investigation of these relations desirable.

Comparison of the rapidity with which carbohydrates and proteids are discharged from the stomach.— The main portion of a diet is more likely to be composed of carbohydrates or proteids or of the two combined than of fats alone. To digest a diet consisting chiefly or even largely of fat is an unusual task for the digestive apparatus. The mechanical treatment of carbohydrates and proteids is, therefore, of more importance practically than the treatment of the fats; and the fact that the stomach is more habituated to the presence of carbo-

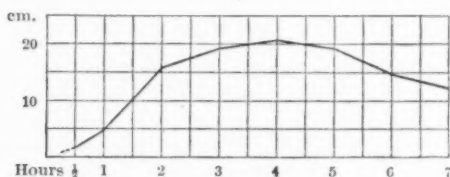


FIGURE 4.—Curve showing average aggregate length in centimetres of the masses of proteid food present in the small intestine at regular intervals for seven hours after feeding various proteid foods. Sixteen cases.

hydrates and proteids in large amounts makes a consideration of the differences in the manner in which these foods are treated of greater significance than a comparison involving the fats.

The curves indicating the carbohydrate and proteid rapidity of discharge from the stomach are strikingly different. At the end of a half-hour eight times as much carbohydrate as proteid has left the stomach; at the end of an hour more than five times as much, and even at the end of two hours, after much carbohydrate food has in all probability been absorbed, considerably more than twice as much carbohydrate as proteid is present in the small intestine (see Fig. 1).¹ This remarkable difference between the carbohydrate and the proteid rapidity of departure from the stomach assumes special significance when the action of the gastric juice on these two food-stuffs is considered. That the carbohydrates, which are not digested

¹ It should be remembered that these comparisons are comparisons of average figures; commonly at the end of a half-hour, and sometimes at the end of an hour the stomach has not released any of its proteid content. Under normal conditions carbohydrates are never thus retained.

by the gastric juice, should begin to leave the stomach soon after being swallowed, and should pass out rapidly into a region where they are digested, while the proteids, which are digested by the gastric juice, should be retained in the stomach for a half-hour, and frequently for an hour, without being discharged in any considerable amount, indicates the presence of an important digestive mechanism.

With the purpose of securing further evidence as to the action of this probable mechanism, various combinations of food-stuffs were fed, and the rate of passage from the stomach studied by the method already described.

THE TREATMENT OF COMBINATIONS OF FOOD-STUFFS BY THE STOMACH.

Effect of feeding carbohydrate first, proteid second. — When different kinds of foods are fed one after another, the first food swallowed fills the antrum pylori and lies along the greater curvature of the stomach, and the later food lies along the lesser curvature and fills the cardiac end.¹ Thus if carbohydrates are fed first and proteids second, the carbohydrates are in contact with the pylorus and predominate in the pyloric end of the stomach, while the proteids are found chiefly in the fundus. Does the presence of proteids in the fundus of the stomach retard the exit of carbohydrates lying near the pylorus? To answer this question, about 12.5 c.c. of crackers and water, mixed with 2.5 grams of subnitrate of bismuth, were fed, and then about 12.5 c.c. of boiled lean beef with 2.5 grams of subnitrate of bismuth. The following results were obtained in four such cases:

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine . . .	3.0	22.0	33.0	30.0	26.0	16.0	9.0	7.0
	4.0	24.0	36.5	26.0	24.0	20.0	17.5	12.0
	8.0	15.5	20.0	26.0	35.0	21.0	19.0	7.5
	3.0	16.0	22.0	24.0	20.0	14.5	13.0	5.0
Average	4.5	19.4	27.9	26.5	26.2	17.9	14.6	7.9

¹ CANNON: This journal, 1898, i, p. 378; also GRÜTZNER: Archives italiennes de biologie, 1901, xxxvi, p. 29.

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Comparison of these figures with the average figures for crackers and water (see p. 397), and for boiled lean beef (see p. 399), proves that the presence of proteids in the cardiac end of the stomach and along the lesser curvature does not materially check the departure of the carbohydrate food lying at the pylorus.

Effect of feeding proteid first, carbohydrate second. — The converse of the relations described in the foregoing paragraph is observed when proteid is fed first, and carbohydrate second. The proteid is then at the pylorus, and the carbohydrate rests chiefly in the fundus of the stomach. Under these circumstances does the presence of the proteid near the pylorus retard the natural early exit of the carbohydrate? To answer this question about 12.5 c.c. of boiled and shredded lean beef with 2.5 grams of bismuth subnitrate were fed, and this was followed by the same amount of moistened crackers with 2.5 grams of bismuth subnitrate. The following results were obtained in four such cases:

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine . . .	0.0	0.0	15.0	23.0	30.0	37.0	30.0	25.0
	3.0	12.0	15.0	18.0	27.0	31.0	26.0	18.0
	6.5	9.0	18.5	16.5	12.5	9.5	4.0	4.0
	0.0	8.5	12.5	19.0	25.0	28.0	25.0	20.0
Average	2.4	7.4	15.2	19.1	23.6	26.4	21.2	16.7

The nearness of these figures to the average figures for boiled lean beef during the first four hours should be noted. And the rate of discharge when carbohydrates are fed first should be compared with the rate when proteids are fed first (Fig. 5). When the crackers are first at the pylorus, the discharge for two hours is almost as rapid as when crackers alone are given. At the end of two hours, however, the curve ceases to follow the normal curve for crackers, — there is a checking of the outgo from the stomach, which is most reasonably explained by assuming that the beef has by that time come to the pylorus in considerable amount, and is as usual passing out slowly. On the other hand, when the beef is first at the pylorus, the curve is in close approximation to the normal curve for beef during the first four hours, and after that time, as the crackers come to

the pylorus in greater amount, the curve continues to rise, while the curve for beef falls. At no time during the first three hours is there half as much food in the small intestine as when crackers alone are fed. It is evident that the presence of proteid near the pylorus distinctly retards the onward passage of carbohydrate food lying in the cardiac end of the stomach.

It is noteworthy that when proteid was given first the stomach still contained considerable food even six hours after feeding,—

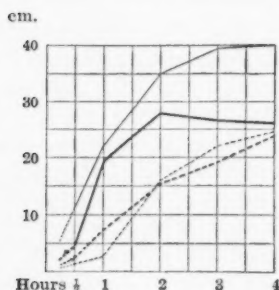


FIGURE 5.—Curve showing average aggregate length of the food-masses in the small intestine at regular intervals for four hours after feeding moistened crackers first, and lean beef second, four cases (heavy continuous line); and after feeding lean beef first, and crackers second, four cases (heavy interrupted line). The light continuous line is the curve for moistened crackers alone; the light interrupted line, the curve for lean beef alone. Four cases are represented in each curve.

a doubling of the time during which carbohydrates alone remain in the stomach. On the other hand, when carbohydrates were fed first, the food was last observed in the stomach at the end of four hours. When the proteid was fed second, therefore, the food was retained in the stomach only about an hour longer than the carbohydrates alone. When one considers the ease with which carbohydrate fermentation may proceed in the cardiac end of the stomach, unchecked for a long time by any acid fluid,¹ the desirability of avoiding such a stasis of the carbohydrate food in the fundus as occurs when proteids lie near the pylorus, becomes at once apparent. Two rational courses seem to be open: either the carbohydrate food should be eaten first, and the proteid second, if the two are taken separately at a meal; or, in case the proteid is eaten first, the carbohydrate should be very carefully chewed. The chewing mixes saliva with

the starchy food, and the food is thereby changed in the cardiac end of the stomach either into sugar, which as a fluid flows downward toward the pylorus, or into dextrin, which undergoes fermentation with difficulty or not at all.²

Effects of mixing in various combinations equal amounts of the three food-stuffs.—Inasmuch as food is generally given as a mixture of the

¹ CANNON: This journal, 1898, i, p. 379.

² CANNON and DAY: This journal, 1903, ix, pp. 407-413.

various food-stuffs, it is important to learn what effect the combining of the various food-stuffs from which characteristic curves have been secured may have upon those curves. For this purpose carbohydrates, fats, and proteids were mixed in equal parts to make 25 c.c. of food, and this mixture, with 5 grams of subnitrate of bismuth, was fed, and the results recorded in the manner already described.

LEAN BEEF AND MOISTENED CRACKERS, IN EQUAL PARTS.								
Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine . . .	7.0	12.0	17.5	23.0	9.0	7.0	6.0	3.5
	0.0	11.0	22.0	25.0	18.5	16.5	15.0	12.5
	8.5	22.0	23.0	19.0	16.0	9.5	7.5	4.5
	11.0	21.0	24.0	26.0	11.5	10.0	9.0	5.0
Average	6.6	16.5	21.6	24.2	13.7 ¹	10.7	9.3	6.3
BOILED HADDOCK AND MASHED POTATO, IN EQUAL PARTS.								
Aggregate length (in centimetres) of food-masses in small intestine . . .	11.0	20.0	36.0	38.0	34.0	17.0	8.5	2.5
	14.0	26.0	29.0	29.0	17.0	8.0	7.5	7.0
	16.0	27.0	35.5	37.0	26.0	19.0	9.0	0.0
	11.0	12.5	28.0	29.0	12.0	8.5	3.0	0.0
Average	13.0	21.4	32.1	33.2 ¹	22.2	13.1	7.0	2.4

¹ That there is more of mixed haddock and potato than of potato alone in the small intestine at the end of three hours is undoubtedly due to the fact that the mixed food passed into the colon one hour later than the potato. That the amount of mixed crackers and lean beef in the small intestine is less than the lean beef alone after three hours is explained by the appearance of the mixed food in the large intestine two hours earlier than the beef appears there when fed by itself.

1. *Effect of mixing carbohydrate and proteid.*—In order to test the effect of mixing carbohydrate and proteid on the rate of discharge from the stomach, equal parts of lean beef and crackers in one series, and equal parts of boiled haddock and mashed potato in another

series, were fed in 25 c.c. amounts. The table on the preceding page presents the results.

In Fig. 6 will be found a comparison between the rate of discharge of the mixed foods, and of the same foods fed separately: in (A) are the curves for crackers (heavy line), and lean beef (light line), and for mixed crackers and beef (dotted line); in (B) are the curves for mashed potato (heavy line), boiled haddock (light line), and mixed potato and haddock (dotted line). Only the changes during the first three hours are taken for consideration, since they are most

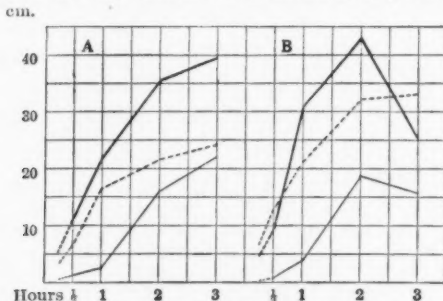


FIGURE 6. — In (A) the average aggregate length of the food-masses in the small intestine during the first three hours after feeding equal parts of moistened crackers and boiled lean beef (dotted line), compared with crackers alone (heavy line), and beef alone (light line). In (B) the average aggregate length of the food-masses in the small intestine during the first three hours, when equal parts of boiled haddock and mashed potato are fed (dotted line), compared with mashed potato alone (heavy line), and haddock alone (light line). Four cases are represented in each curve.

significant in judging the rapidity of discharge from the stomach. In both cases here presented, the amount of the mixed food in the small intestine at the end of a half-hour is nearer the carbohydrate than the proteid figure; indeed, in the case of mixed haddock and potato, there had left the stomach during the first half-hour a little more of the mixed food than of the potato alone. But the above tables and the accompanying curves (Fig. 6) clearly show that in general when carbohydrates and proteids are mixed in equal parts, the rate of discharge through the pylorus is intermediate; the mixed food does not leave the stomach so slowly as the proteids, nor so rapidly as the carbohydrates.

2. *Effect of mixing fat and proteid.* — Boiled lean beef and beef suet served for the observations on the effect of mixing fat and proteid. These substances were mixed in equal amounts, and fed in the manner already described, with the following results:

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine . .	0.0	0.0	6.5	9.0	11.5	15.0	12.5	9.5
	0.0	0.0	5.0	8.0	10.5	7.5	6.5	7.5
	0.0	2.5	10.0	12.0	17.5	19.5	14.5	10.5
	0.0	5.5	14.0	18.5	21.5	16.5	15.0	13.0
Average	0.0	2.0	8.8	11.8	15.2	14.6	12.1	10.1

Comparison of these results with the average figures for beef suet and for lean beef (Fig. 7) reveals at once that when the two are mixed in equal parts, the rate of discharge from the stomach is diminished to a degree below that of either the lean beef or the suet fed by itself. In other words, the presence of the fat causes proteid to leave the stomach even more slowly than the proteid by itself would leave. This conclusion was corroborated by feeding haddock and mutton fat in equal amounts, in which case at the end of two hours there was in the small intestine only two-thirds as much of the mixed food as of the haddock when it was given alone. In all probability the long delay of an hour or an hour and a half before the initial passage of food from the stomach, noted in an earlier research when salmon was fed, was due to the presence in salmon of more than half as much fat as proteid.

3. *Effect of mixing fat and carbohydrate.*—Mashed potato and mutton fat, and moistened crackers and beef suet, mixed in each case in equal parts, were used in studying the effect of combining carbohydrates and fats. The table on the following page presents the results of these observations.

In both series of observations the passage of the mixed food from the stomach is more rapid at first than the normal rate for the carbohydrate used (see Fig. 8). Very soon, however, the fats have a

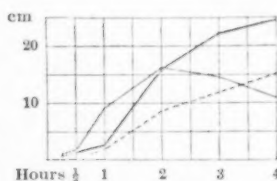


FIGURE 7.—Curves showing the average aggregate length of the masses of lean beef (heavy line), of beef suet (light line), and of beef and beef suet mixed in equal parts (dotted line), in the small intestine during the first four hours after feeding. Four cases are represented in each curve.

retarding effect on the outgo of the carbohydrate from the stomach, so that the curve for the mixed food-stuffs, after the first hour, ceases to rise, and never even approximates the height of the carbohydrate curve. The failure of the curve for mixed crackers and beef suet to rise might in part be ascribed to the remarkably early discharge of the contents of the small intestine into the colon; but the curve for

MASHED POTATO AND MUTTON FAT.								
Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length (in centimetres) of food-masses in small intestine . . .	14.0	19.0	20.0	21.0	19.5	15.0	14.0	8.0
	11.0	18.0	19.0	20.0	19.0	13.0	9.5	9.5
	13.0	21.0	20.0	18.5	16.0	14.0	14.0	12.5
	14.5	22.0	19.0	20.0	24.0	16.0	10.5	9.0
Average	13.1	20.0	19.5	19.9	19.6	14.5	12.0	9.7
MOISTENED CRACKERS AND BEEF SUET.								
Aggregate length (in centimetres) of food-masses in small intestine . . .	18.0	28.5	15.0	9.0	6.5	3.5	0.0	0.0
	22.0	30.0	32.0	18.0	15.0	13.5	9.0	5.5
	24.0	27.0	21.0	20.0	15.0	4.0	4.0	2.5
	10.5	22.5	18.0	16.0	10.5	10.0	7.0	4.5
Average	18.6	27.0	21.5	15.7	11.7	7.7	5.0	3.1

mixed potato and mutton fat remains low for more than three hours without that factor becoming operative in more than one case. It is reasonable; therefore, to conclude that the mixture of fat in large amount (50 per cent) with carbohydrate has the same effect, though not to so great a degree, as the mixture of fat with proteid, — namely, the fat causes the outgo of the carbohydrate and proteid from the stomach to be slower than the outgo of either of the two foods fed separately.

EFFECT OF INCREASING THE AMOUNT OF FOOD.

All the observations detailed thus far have been made in each instance on 25 c.c. of food. It is desirable, especially in the case of carbohydrates and of proteids, to know if the feeding of larger amounts of food will result in curves differing from those secured when smaller amounts are fed. Boiled rice, representing carbohydrates, and lean beef, representing proteids, were fed in twice the amount previously given. The results are seen in the following table :

BOILED RICE. 50 c.c.								
Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate lengths (in centimetres) of food-masses in small intestine . . .	19.0	31.0	41.0	38.0	36.0	28.0	21.0	13.0
	12.0	25.0	40.0	40.0	38.0	36.0	24.0	13.0
	32.0	48.0	40.0	23.0	17.0	8.0	6.5	4.5
	22.0	44.0	50.0	28.0	27.0	17.0	14.0	12.0
Average	21.2	37.0	42.7	32.2	29.5	22.2	16.4	10.6
LEAN BEEF, BOILED. 50 c.c.								
Aggregate lengths (in centimetres) of food-masses in small intestine . . .	0.0	0.0	11.0	18.0	24.0	21.0	14.5	14.0
	0.0	0.0	4.0	19.0	27.0	29.0	23.5	16.5
	0.0	0.0	6.0	19.0	28.0	21.5	16.0	12.5
	0.0	0.0	10.5	15.0	20.0	17.0	15.0	14.0
Average	0.0	0.0	7.9	17.7	24.7	22.1	17.2	14.2

In Fig. 9 are the curves of each of these food-stuffs in the 25 c.c. and in the 50 c.c. amounts. The rapid rate of carbohydrate discharge, and the slow rate of proteid discharge, have both been increased. At the end of an hour over 25 per cent more carbohydrate has left the stomach when the amount fed is 50 c.c. than when the amount fed is 25 c.c. And with the proteid food, when the amount was 50 c.c., nothing left the stomach for an hour, and at the end of

two hours only half as much had left as when the amount was 25 c.c. Increasing the amount of food, therefore, increases the rate of discharge of carbohydrates from the stomach, and retards the out-going of the proteids.

THE PASSAGE OF THE DIFFERENT FOOD-STUFFS THROUGH THE SMALL INTESTINE.

The chyme emerges from the stomach in small amounts. Usually there is an accumulation of these small discharges into a slender mass in the duodenum before the food is further advanced in the intestine. But as time goes on, these masses tend to gather into longer

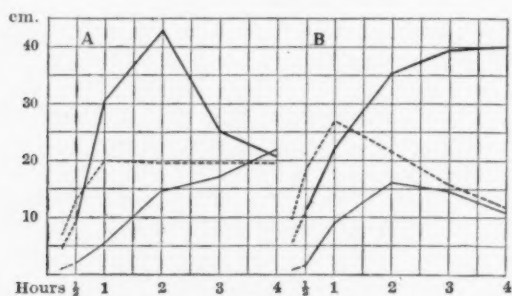


FIGURE 8. — (A) Curves showing the average aggregate length of the masses of mashed potato (heavy line), of mutton fat (light line), and of mixed potato and mutton fat (dotted line), in the small intestine during the first four hours after feeding. (B) Similar curves for crackers (heavy line), beef suet (light line), and mixed crackers and beef suet (dotted line). Four cases are represented in each curve.

masses, so that at the end of three or four hours the food in the coils is often largely collected in a few unbroken strings. This condition occurs earlier with carbohydrate than with proteid food (see Fig. 1, A and B).

In the small intestine the food is mixed with the intestinal digestive secretions, exposed to the absorbing intestinal wall, and from time to time moved forward into new areas of intestinal activity. The mixing of the food with the secretions, and the exposing of the food for absorption, are functions performed in the rhythmic segmentation of the food by circular constrictions of the gut; the forward movement is the result of ordinary peristalsis; but at any moment most of the food in the intestine lies undisturbed.¹ In studying the

¹ CANNON: This journal, 1902, vi, p. 263.

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passage of the different food-stuffs through the small intestine, therefore, attention must be paid to the process of segmentation and to the rate of passage from the stomach to the large intestine.

Rhythmic segmentation with the different food-stuffs.—The segmentation of the food occurs with all three kinds of food-stuffs. The frequency with which it is seen at the regular times of observation adhered to throughout this research has varied, as shown in the following table. The figures represent the number of cases in which segmentation was observed at the hours indicated.

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Carbohydrates, 16 cases .	7	11	11	8	9	6	0	0
Fats, 16 cases	3	6	5	4	4	4	1	1
Proteids, 16 cases	0	6	9	9	8	5	3	2

Comparison of these figures with the average figures for the intestinal content of the different foods at the regular times of observation (see pp. 395, 397, and 399) shows that the frequency with which segmentation occurs corresponds roughly to the amount of food present in the intestine. Thus, when carbohydrates are fed, segmentation is observed frequently, but more frequently during the first hours than later. Similar comparisons of the fats and proteids show that, as a rule, when much food of any kind is present in the intestine, segmentation is more likely to be seen than when a small amount is present.

The amount of work the musculature of the intestine performs with the different foods is indicated better by the total length of the masses segmented, than by the frequency of the occurrence of that process. The length of all the segmented masses in the small intestine in the sixteen cases of each food-stuff is as follows:

Hours after feeding .	$\frac{1}{2}$	1	2	3	4	5	6	7	Total.
Carbohydrates . .	40.3	89.4	132.1	76.6	90.3	35.6	0.0	0.0	=464.3
Proteids	0.0	34.1	54.0	51.6	46.4	28.7	27.5	12.7	=248.0
Fat	20.0	39.0	22.0	46.4	14.5	20.7	5.3	3.5	=171.4

These figures do not, of course, record the total segmentation taking place during a complete digestive period, but they indicate the amount of activity which may be expected at given intervals during the first seven hours after feeding. These figures also indicate that the muscular energy expended on carbohydrates in the seven hours far exceeds that expended either on proteids or fats. The longer presence of proteids and fats in the small intestine, because of the slower discharge of these foods from the stomach, would later compensate in part for the relatively small amount of segmentation during the first seven hours; but at the end of seven

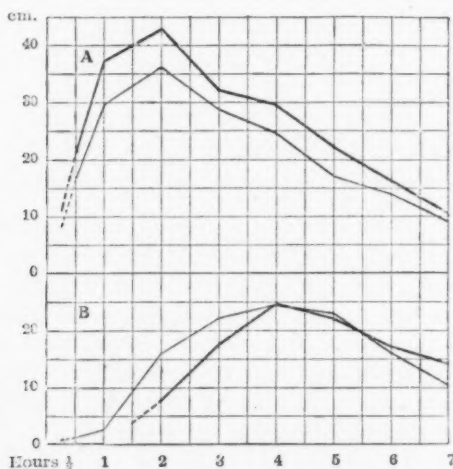


FIGURE 9. — (A) Curves showing the average aggregate length of the food-masses in the small intestine when 50 c.c. boiled rice are fed (heavy line), and when 25 c.c. are fed (light line). (B) Similar curves for 50 c.c. boiled lean beef (heavy line), and for 25 c.c. lean beef (light line). Four cases are represented in each curve.

hours the proteid content of the small intestine is almost the same as the carbohydrate content (compare Figs. 3 and 4), and so slight is the segmenting activity in the presence of fats that, should their rate continue, — and that seems hardly probable when the dwindling of the segmentation during the last hours is considered, — they must remain in the small intestine for almost nineteen hours to produce an activity equal to that produced by carbohydrates. It appears very likely, therefore, that in the small intestine a much greater amount of muscular activity in the form of rhythmic segmentation is provoked

by carbohydrate food than by either of the other two food-stuffs. It is noteworthy that the carbohydrates, which cause the greatest amount of muscular activity in the small intestine, cause the least amount in the stomach.¹

Rate of segmentation with the different food-stuffs. — In previous observations on segmentation in the small intestine, the rate was found to vary from eighteen to thirty movements per minute when salmon was fed. Observations with other foods reveal no greater variation than that seen with salmon, and there was nothing characteristic in these variations.

Rate of passage of the different food-stuffs through the small intestine. — In studying the passage of food through the small intestine of a woman with a fistula at the ileo-colic junction, MacFadyen, Nencki, and Sieber² noted a considerable variation in the time between the ingestion of the food and its appearance at the fistula.

For example, peas first arrived at the colon on one occasion two and a quarter hours, and on another occasion five and a quarter hours, after being eaten. Demarquay,³ who studied a case similar, but apparently less normal, reports also a wide variation in the time of the first appearance of food at the fistula.

The method used in this research does not permit a statement of the moment when food first entered the colon; all that can be reported is the first observation when food was seen in the colon. Inasmuch as the observations were an hour apart, the results, except

¹ Curiously the proteid (egg-albumin), which leaves the stomach with carbohydrate rapidity, more nearly resembles the carbohydrates than the proteids in undergoing much segmentation by the intestines. The following figures, giving the total length of the segmenting masses in *five* cases in which egg-albumin was fed, illustrate the abundant segmentation to which this food is subjected :

Hours after feeding	$\frac{1}{2}$	1	2	3	4	5	6	7
	0.0	19.8	25.2	4.4	8.7	0.0	7.7	0.0

It was the frequency with which segmentation takes place when egg-albumin is fed, compared with the frequency of segmentation observed in a few cases in which starch paste, and others in which olive oil was administered, that led to an erroneous statement that proteids were more provocative of segmentation than fats or carbohydrates. See Proceedings of the American Physiological Society, This journal, 1902, viii, p. xxii.

² MACFADYEN, NENCKI, and SIEBER: *Journal of anatomy and physiology*, 1891, xxv, p. 393.

³ DEMARQUAY: *L'union médicale*, 1874, xviii, p. 906.

Hours after feeding . . .		2	3	4	5	6	7	8 ¹
Carbohydrates.	Mashed potato	1	3
	Moistened crackers	3	..	1	..
	Rice, boiled	2	2
	Starch pudding	1	2	1
Total		1	6	4	4	..	1	..
Proteids.	Fowl	2	1	1
	Haddock, boiled	1	2	1
	Lean beef, boiled	3	1	..
	Fibrin	1	2	..	1
Total	1	2	2	7	2	2
Fats.	Tallow	2	1	1
	Beef suet	2	..	2
	Pork fat	1	1	1	1	..
	Mutton fat	3	..	1	..
Total	2	3	7	2	2	..

¹ In four cases no food had appeared in the colon at the end of seven hours. In this table, these cases are regarded as coming in an eight-hour class.

NUMBER OF CASES IN WHICH, AT VARIOUS HOURS AFTER FEEDING, FOOD WAS FIRST OBSERVED IN THE LARGE INTESTINE.—*continued.*

Hours after feeding . . .		2	3	4	5	6	7	8
Combinations.	Mutton fat and potato	1	2	..	1	..
	Beef suet, and crackers . .	2	1	1
	Lean beef and crackers	1	3
	Haddock and potato	1	2	1
	Haddock and mutton fat	1	2	1	..
	Lean beef and beef suet	1	1	2
	Crackers, first; beef, second	3	1
	Beef, first; crackers, second	..	1	1	2	..
Rice, 50 c.c.		1	2	..	1
Beef, 50 c.c.	2	2

The variation reported in cases of low intestinal fistula in human beings appears also in these tabulated results. Although the mean time after eating at which the food reaches the colon is about four hours for carbohydrates, about six hours for proteids, and about five hours for fats, the divergence from the mean in each of the three classes is considerable. It is interesting to note that the divergence among the carbohydrates is chiefly due to one food, the moistened crackers. The long stay of this food in the small intestine accounts for the prolonged high curve for crackers (Fig. 6, *A*), so different from the quick rise and rapid fall of the curve usual with carbohydrates (Fig. 6, *B*). Among proteids also the divergence from the mean is most marked in the case of one food,—the boiled haddock. This food arrives at the colon about two hours earlier than most of the other proteids, and its relatively rapid passage through the small intestine explains the exceptional early decline of the curve of the intestinal content in the case of this proteid (see Fig. 6, *A* and *B*, light lines).

The two combinations, crackers and lean beef, and crackers and beef suet, may be compared with crackers alone, as to the time required to traverse the small intestine; both combinations pass through the small intestine more rapidly than this carbohydrate food

by itself. Even feeding crackers before feeding lean beef seems to make the crackers reach the colon earlier. On the other hand the combination of potato with haddock and with mutton fat apparently causes a checking of the rate of passage of this carbohydrate from the stomach to the colon. Hence no conclusion can be drawn from these cases.

The combination of proteids and fats, in most instances, has the effect of delaying the appearance of the mixed foods in the colon to a time later than that recorded when the fats alone were fed.

The average figures for carbohydrates, proteids, and fats in the above table demonstrate that, as a rule, the carbohydrates reach the large intestine about one hour before the fats and about two hours before the proteids. As there is no considerable passage of proteid food from the stomach into the small intestine until about an hour after the carbohydrate food has begun to leave the stomach in large amount, the difference of an hour still remains between the carbohydrate and proteid stay in the small intestine. It is probable that, in general, the proteids pass from the stomach to the large intestine more slowly than do the carbohydrates, while the fats have a rate intermediate between these two.

SUMMARY.

Fat, carbohydrate, and proteid foods, uniform in amount (25 c.c.) and consistency, were mixed with a small amount of subnitrate of bismuth and fed to cats deprived of food for at least twenty-four hours. The rate of gastric peristalsis observed by means of the Röntgen rays was usually slower for fats (5.2 waves per minute) than for carbohydrates (5.8 waves per minute), but the variation was so great as to make a more definite statement unsafe.

At regular intervals for seven hours after feeding, the shadows of the intestinal contents were traced on transparent paper by means of a fluorescent screen and the Röntgen rays. Since the intestinal contents vary only slightly in diameter, the aggregate length of the shadows can be taken to indicate the relative amount of food present in the small intestine at various intervals, and in various animals at the same interval after feeding. In the early stages of intestinal digestion, before much absorption has occurred, the aggregate length of the shadows at different intervals indicates the rate of discharge from the stomach.

Fats remain long in the stomach. The discharge of fats begins slowly and continues at nearly the same rate at which the fat leaves the small intestine by absorption and by passage into the large intestine. Consequently there is never any great accumulation of fat in the small intestine.

Carbohydrate foods begin to leave the stomach soon after their ingestion. They pass out rapidly, and at the end of two hours reach a maximum amount in the small intestine almost twice the maximum for proteids, and two and a half times the maximum for fats, both of which maxima are reached only at the end of four hours. The carbohydrates remain in the stomach only about half as long as the proteids.

Proteids frequently do not leave the stomach at all during the first half-hour. After two hours they accumulate in the small intestine to a degree only slightly greater than that reached by carbohydrates an hour and a half earlier. The departure of proteids from the stomach is therefore slower at first than that of either fats or carbohydrates. An exception to this general statement was found in egg-albumin, which, both in its natural state and in coagulated form, was discharged from the stomach at about the carbohydrate speed.

When carbohydrates are fed first and proteids second, the presence of proteids in the cardiac end of the stomach does not materially check the departure of the carbohydrate food lying at the pylorus; but the presence of proteids near the pylorus, when proteids are fed first and carbohydrates second, markedly retards the onward passage of the carbohydrates which under these circumstances predominate in the cardiac end of the stomach.

When carbohydrates and proteids are mixed in equal parts, the mixed food does not leave the stomach so slowly as the proteids, nor so rapidly as the carbohydrates, — the discharge is intermediate in rapidity.

In a mixture of fats and proteids in equal parts, the presence of the fat causes the proteid to leave the stomach even more slowly than the proteid by itself. Fat mixed with carbohydrate in equal amounts also causes the carbohydrates to pass the pylorus at a rate slower than their normal.

Doubling the amount of carbohydrate food (50 c.c. instead of 25 c.c.) increases the rapidity of the carbohydrate outgo from the stomach during the first two hours; whereas doubling the amount of proteid food strikingly delays the initial discharge of proteid from the stomach.

The process of rhythmic segmentation is seen with all three kinds of food-stuffs, and the frequency of its occurrence corresponds roughly to the amount of food present in the intestine; a measurement of the length of the segmenting masses in a given number of cases shows that at the regular times of observation, during the first seven hours after feeding, the amount of segmenting activity in the presence of carbohydrates was much greater than in the presence of either fats or proteids. Egg-albumin is excepted in this general statement.

The interval between the feeding and the appearance of food in the large intestine is variable, but the mean for carbohydrates is about four hours, for proteids about six hours, and for fats about five hours. After time is allowed for the later start of proteids from the stomach, there still remains a probability that the proteids pass through the small intestine more slowly than do the carbohydrates.

The discussion of the observations here presented, and the relation of these observations to the work of other investigators, is deferred to a later paper. This paper will report experiments undertaken to explain the characteristic differences of treatment of the food-stuffs, which have been described in the foregoing pages.



THE TOXIC AND ANTI-TOXIC ACTION OF SALTS.

By A. P. MATHEWS.

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SINCE Ringer's¹ observations on the action of potassium and calcium salts on the heart, it has been known that the physiological action of certain salts could be modified by other salts. Ringer² discovered also that this antagonism was not confined to electrolytes, but that the action of veratrin, one of the most powerful of protoplasmic poisons, could be neutralized very completely by potassium and calcium salts.² This antagonism of common electrolytes to so powerful a drug is of importance theoretically, for the light it may throw on the means of action of drugs on protoplasm, and practically, since it suggests the possibility of alterations in the toxic value of poisons by salts.

To discover if other drugs could be neutralized by calcium salts, Mr. Rothrock, a medical student, examined under my direction, two years ago, the drug digitalis. He found at least a partial antagonism between digitalis and calcium chloride, but as his results were not entirely convincing they were not published. I obtained a better result with physostigmine and these results were extended and published by S. A. Matthews and O. S. Brown.³ There is, therefore, little doubt that the whole group of drugs which act like barium chloride, namely, suprarenal extract, physostigmine, veratrin (?), and digitalis may be largely or completely antagonized by calcium chloride. Recently McCallum⁴ has found an antagonism between cascara and calcium for the intestinal movements, and Hektoen has shown that the activity of some bacterial hæmolysins is greatly altered by inorganic salts present in the solutions.

¹ RINGER: *Journal of physiology*, 1884, v, p. 247.

² RINGER: *Ibid.*, p. 352.

³ MATTHEWS and BROWN: *This journal*, 1904, xii, p. 173.

⁴ MACCALLUM: *Journal of experimental zoölogy*, 1904, i, p. 179.

These facts show that the same kind of an antagonism exists between salts and drugs not ordinarily classed as electrolytes as between two electrolytes. This fact, in my opinion, supports the conclusion of a former paper¹ that both electrolytes and non-electrolytes act physiologically by their dissociation products. The anti-toxic action of the toxins may therefore be most easily approached by a study of the anti-toxic relations of electrolytes.

I have studied the antagonistic action of salts on the developing eggs of the fish *Fundulus heteroclitus*. While I have not yet been able to determine fully the nature of the anti-toxic action, I have decided to publish the results, for the reason that they necessitate certain changes in the conclusions drawn by Loeb from a similar study on the same form.

Loeb,² extending Ringer's observations on the action of solutions on developing ova, found that pure solutions of sodium, potassium, lithium, and ammonium chlorides were fatal to *Fundulus* embryos; but that the addition of small amounts of calcium, magnesium, barium, strontium, manganese, cobalt, and other salts would offset the poisonous action and permit development to take place. This fact may be easily confirmed by any one, and my own observations are in accord with it. My results, however, are not in accord with Loeb's several explanations of the fact.

In his first paper Loeb states that the anti-toxic power depends upon the valence of the cation. A solution containing only monovalent cations was poisonous, but the addition of any salt containing a bivalent cation, or the addition of a small quantity of a salt with a trivalent cation, neutralized this action. There was hence a toxic and anti-toxic action between monovalent and polyvalent cations.³ This action concerned only the cations, since the same results were obtained with chlorides, nitrates, and acetates. That the toxic and anti-toxic action depended upon the valence of the cation and not upon its chemical composition was shown by the fact that most bivalent metals had the anti-toxic action which was about equally powerful in all.⁴ It will be perceived that the correctness of the conclusions depends upon the truth of the last statement, since if it happens that the anti-toxic power of bivalent metal salts is not the same,

¹ MATHEWS: This journal, 1904, x, p. 290.

² LOEB: Archiv für die gesammte Physiologie, 1901, lxxxviii, p. 68.

³ LOEB: *Loc. cit.*, p. 74.

⁴ LOEB: *Loc. cit.*, p. 72.

but varies widely, there would be as much reason for referring their action to their chemical nature as to their valence.

Loeb's results indeed contained a fact which threw serious doubt on the truth of this statement. No anti-toxic effect could be obtained with mercury or copper salts; nor would ferric chloride neutralize. There was hence a marked difference between some of these metals with the same valence. To explain this it was assumed that there is some sort of a specific toxicity on the part of ions which might quite mask their anti-toxic powers. This assumption is plainly incompatible with the primary conclusion that valence is the determining factor in toxicity.

These results offered a certain parallelism with Hardy's observations on the precipitation of albumin which showed that the valence of the anion or cation determined its precipitating power. It was suggested that the salts acted toxically by altering the state of aggregation of the colloids in the cell.

In a second paper¹ on the same subject, a totally different explanation of the facts is given. In this paper it was assumed that the poisonous action of sodium chloride was due to the predominance of chlorine over sodium. In calcium chloride the calcium predominated. The negative ion was supposed to be toxic, the positive anti-toxic. In thus concluding that chlorine was toxic, and the cation anti-toxic, the results were brought into harmony with Pauli's² interpretation of the coagulation of egg-albumin by salts.

In a subsequent paper,³ however, this view was in turn abandoned, and a return made to the original conclusion that a toxic and anti-toxic action exists between mono- and bi-valent cations, although the predominant importance of valence was no longer so strongly insisted upon, and the statement is made that in acids, at least, the anion is not altogether negligible.

The matter was thus left in an unsatisfactory state, and a reinvestigation seemed desirable.

I first investigated the cause of the toxic action of salts with particular regard to the question of valence. If valence was not important in determining toxicity, it could hardly be of importance in determining anti-toxic action. The results of this investigation have already been published.⁴ They showed in the clearest manner that

¹ LOEB: This journal, 1902, vi, p. 429.

² PAULI: Archiv für die gesammte Physiologie, 1899, lxxviii, p. 315.

³ LOEB and GIES: Archiv für die gesammte Physiologie, 1902, xciii, p. 246.

⁴ MATHEWS: This journal, 1904, x, p. 290.

valence, as such, is of very little or no importance in determining toxicity. For example, strontium chloride and sodium chloride are almost equally toxic in equivalent solutions. Manganese chloride is far more toxic, zinc enormously more toxic, while the univalent silver is the most poisonous of all. It is clear that if valence is of any importance whatever, it is negligible compared to the importance of some other factor. That other factor was shown to be the ionic potential or the decomposition tension of the salt. While there were certain exceptions to the rule that non-poisonous salts had a high decomposition tension, and poisonous salts a low decomposition tension, the general correspondence was unmistakable. For some of the exceptions also, explanations compatible with the conclusion have since been found. Valence is not, therefore, of first importance in determining toxicity.

An investigation was also started to determine whether valence was of importance in the precipitation of colloidal solutions, to see whether the behavior of colloidal solutions really lent support to Loeb's conclusions. The results of this study are not complete, but it is clear, both from the data already obtained, and the results of others, that some other factor than valence is of great importance in the precipitation of colloidal solutions. Indeed, it is probable that valence, as such, plays a very subordinate part in this process. Hardy's albumin solution, for example, is more easily precipitated by monovalent silver than by bivalent calcium. The monovalent metals, sodium, potassium, and lithium, are all alike in having a very low ionic potential. Their low precipitating powers may as readily be referred to this as to their being monovalent. The greater precipitating power of ferric over ferrous chloride is in my opinion undoubtedly correlated with its ionic potential, rather than with its change in valence. While, therefore, a positive statement cannot be made, the facts strongly indicate that in precipitation also, valence, as such, is relatively unimportant.

Having shown that the toxic action of salts is determined by their decomposition tension, and not by the valence of their ions, I next tested the truth of the statement that all bivalent cations were of equal anti-toxic value. To determine whether this was the case, the toxicity of $\frac{1}{8}$ N sodium chloride and sodium nitrate containing known quantities of magnesium, barium, and other salts was tested, and the minimum concentration of the bivalent ions necessary to neutralize the toxic action of sodium chloride was determined in each case.

TABLE I.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
NaCl	$\frac{5}{8}$ M	MgCl ₂	$\frac{100}{4000}$	0	NaNO ₃	$\frac{5}{8}$ M	CaCl ₂	$\frac{100}{12000}$	0
"	"	"	$\frac{100}{1600}$	0	"	"	"	$\frac{100}{4000}$	12
"	"	"	$\frac{100}{800}$	0	"	"	"	$\frac{100}{2000}$	75
"	"	"	$\frac{100}{333}$	0	NaCl	"	"	$\frac{100}{4000}$	9
"	"	"	$\frac{100}{400}$	0	"	"	"	$\frac{100}{1600}$	60
"	"	"	$\frac{100}{288}$	0	"	"	"	$\frac{100}{800}$	85
"	"	"	$\frac{100}{200}$	5	"	"	"	$\frac{100}{400}$	88
"	"	"	$\frac{100}{160}$	4	NaNO ₃	$\frac{5}{8}$ M	Co(NO ₃) ₂	$\frac{100}{8000}$	0
"	"	"	$\frac{100}{100}$	8	"	"	"	$\frac{100}{3200}$	0
"	"	"	$\frac{100}{80}$	50	"	"	"	$\frac{100}{2255}$	0
"	"	"	$\frac{100}{56}$	85	"	"	"	$\frac{100}{1835}$	0
NaCl	$\frac{5}{8}$ M	SrCl ₂	$\frac{100}{1600}$	0	"	"	"	$\frac{100}{800}$	61
"	"	"	$\frac{100}{800}$	0	"	"	"	$\frac{100}{400}$	80
"	"	"	$\frac{100}{333}$	14	NaNO ₃	$\frac{5}{8}$ M	NiCl ₂	$\frac{100}{10000}$	0
"	"	"	$\frac{100}{400}$	60	"	"	"	$\frac{100}{5000}$	0
"	"	"	$\frac{100}{288}$	85	"	"	"	$\frac{100}{2666}$	0
"	"	"	$\frac{100}{200}$	80	"	"	"	$\frac{100}{800}$	4
NaNO ₃	$\frac{5}{8}$ M	BaCl ₂	$\frac{100}{10000}$	0	"	"	"	$\frac{100}{400}$	0
"	"	"	$\frac{100}{4000}$	0	"	"	"	$\frac{100}{200}$	0
"	"	"	$\frac{100}{2666}$	0	LiCl	$\frac{5}{8}$ M	SrCl ₂	$\frac{100}{1000}$	0
"	"	"	$\frac{100}{2000}$	0	"	"	"	$\frac{100}{500}$	3
"	"	"	$\frac{100}{1600}$	10	"	"	"	$\frac{100}{166}$	30
"	"	"	$\frac{100}{800}$	25	LiCl	$\frac{5}{8}$ M	MgCl ₂	$\frac{100}{800}$	0
NaCl	"	"	$\frac{100}{1600}$	40	"	"	"	$\frac{100}{160}$	0
"	"	"	$\frac{100}{800}$	90	"	"	"	$\frac{100}{32}$	30
"	"	"	$\frac{100}{400}$	92					

TABLE I. — Continued.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
$\text{NaC}_2\text{H}_3\text{O}_2$	$\frac{5}{8} n$	BaCl_2	$\frac{m}{2000}$	0	$\text{NaC}_2\text{H}_3\text{O}_2$	$\frac{5}{8} n$	CaCl_2	$\frac{m}{2000}$	30
"	"	"	$\frac{m}{1333}$	35	"	"	"	$\frac{m}{2000}$	62
"	"	"	$\frac{m}{800}$	60	"	"	"	$\frac{m}{1600}$	91
"	"	CaCl_2	$\frac{m}{8000}$	0	"	"	"	$\frac{m}{1000}$	100
"	"	"	$\frac{m}{4000}$	6	"	"	"	$\frac{m}{333}$	100

The foregoing experiments show in the clearest way the wide differences of anti-toxic action between even such closely allied salts as those of magnesium, calcium, strontium, and barium. To permit any embryos to develop in $\frac{5}{8} n$ sodium chloride or sodium nitrate solutions, magnesium chloride must be present in a concentration of $\frac{m}{2000}$; strontium chloride in $\frac{m}{533}$; barium chloride in $\frac{m}{1600}$; and calcium chloride in something less than $\frac{m}{4000}$. Taking the molecular anti-toxic action of magnesium chloride as unity, the following values are obtained:

$$\text{MgCl}_2 = 1.0$$

$$\text{SrCl}_2 = 3.33$$

$$\text{BaCl}_2 = 10.0$$

$$\text{Co}(\text{NO}_3)_2 = 6$$

$$\text{CaCl}_2 = 25$$

$$\text{NiCl}_2 = \text{Practically nothing.}$$

For sodium nitrate, for lithium chloride, potassium chloride, sodium acetate, and ammonium chloride, the comparative anti-toxic values are the same as those given for sodium chloride. Nickel chloride is almost inert anti-toxically, in this respect differing greatly from cobalt nitrate and chloride. Although Loeb reports an anti-toxic action of lead acetate, my own results with this salt were negative. Confirming his observations, I found it impossible to get any neutralization with copper or mercuric chlorides. With ferrous chloride, a counteraction against potassium chloride was obtained in solutions stronger than $\frac{m}{1600}$.

The wide variations in anti-toxic power thus existing between closely related salts, having cations of the same valence, strongly indicates that valence is not the sole factor in the anti-toxic action, if indeed it has any importance whatever.

The next step was to determine whether the anti-toxic action was between the cations and independent of the anions.¹ Loeb's reason for concluding that the anti-toxic action was between the cations only, was the fact that calcium chloride neutralized about equally well the poisonous action of sodium chloride, nitrate, and acetate. As these anions are alike in having a high solution tension, I have examined a number of other salts of which the anion is univalent, but where the ionic potential is quite different.

The experiment already quoted showed that calcium chloride would neutralize sodium acetate better than sodium nitrate or chloride; with the acetate one hundred per cent of embryos may easily be developed. The observations already published, showing that the toxic action was a function of both ions, clearly showed that Loeb's statement must be incorrect. The specific experiments recorded in Table II were tried.

These experiments demonstrate conclusively that both ions of the toxic salt are involved in the anti-toxic action, and that this cannot accordingly be referred to either ion alone. While slight differences exist in the amount of calcium chloride necessary to neutralize the chlorides, nitrates, bromides, and acetates, at least twice as much must be used for each molecule of sulphate, and for the bromates nearly eighty times as much is needed as for the chlorides. It is impossible to neutralize the sulphocyanate with any concentration I have tried, and this in spite of the fact that this salt is only a little more poisonous than the chloride. The iodide also is neutralized with difficulty. There is, therefore, no question but that toxic and anti-toxic action involves the anion as well as the cation.

A necessary corollary of this conclusion is the conclusion that the anion of the anti-toxic salt is also of importance, and cannot be disregarded as was done by Loeb. It is clear that if a calcium salt of a different anion from chlorine is used, the chlorine or other anion introduced with the calcium will be distributed between the anti-toxic and toxic salt. If, for example, calcium bromate is introduced, an interchange will take place between the sodium and calcium, so that sodium bromate will be formed.

Specific evidence of the importance of the anion was obtained by comparing the anti-toxic action of barium chloride and barium hydrate on sodium acetate (Table III).

¹ In LOEB'S second explanation the anions were supposed to be the toxic agent. I have not considered this second explanation, for the reason that he repudiates it later.

TABLE II.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
KCyS	$\frac{1}{100}$	0	NaBrO ₃	$\frac{5}{100}$	0
"	"	CaCl ₂	$\frac{1}{800}$	0	"	"	CaCl ₂	$\frac{1}{500}$	0
"	"	"	$\frac{1}{175}$	0	"	"	"	$\frac{1}{200}$	0
"	"	"	$\frac{1}{240}$	0	"	"	"	$\frac{1}{104}$	0
"	"	"	$\frac{1}{320}$	0	Na ₂ SO ₄	$\frac{5}{100}$	0
"	"	"	$\frac{1}{160}$	0			CaCl ₂	$\frac{1}{3333}$	0
"	"	"	$\frac{1}{24}$	0			"	$\frac{1}{2000}$	0
KCl	$\frac{1}{6}$	10			"	$\frac{1}{1000}$	20
"	"	CaCl ₂	$\frac{1}{8000}$	5			"	$\frac{1}{800}$	87
"	"	"	$\frac{1}{4000}$	5	KI	$\frac{5}{14}$	CaCl ₂	$\frac{1}{640}$	30
"	"	"	$\frac{1}{1600}$	20			"	$\frac{1}{128}$	50
KBrO ₃ *	$\frac{1}{8}$	5			"	$\frac{1}{64}$	50
"	"	CaCl ₂	$\frac{1}{240}$	0			"	"	"
"	"	"	$\frac{1}{320}$	5			"	"	"
"	"	"	$\frac{1}{160}$	0					
"	"	"	$\frac{1}{35}$	30					

* For the control but a single experiment was tried. One egg developed in 20.

Barium hydrate is fatal by itself in concentrations stronger than $\frac{1}{400}$. The marked differences in anti-toxic action between the hydrate and chloride clearly show the importance of the anion of the anti-toxic salt.

The comparative anti-toxic action of calcium and other salts with bivalent cations on different monochlorides also indicates that something other than valence is the determining factor. The chlorides of sodium, potassium, ammonium, and lithium are all monovalent, and

TABLE III.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
$\text{NaC}_2\text{H}_3\text{O}_2$	$\frac{5}{8} m$	0	$\text{NaC}_2\text{H}_3\text{O}_2$	$\frac{5}{8} m$	Ba(OH)_2	$\frac{20}{10000}$	0
"	"	BaCl_2	$\frac{20}{10000}$	0	"	"	"	$\frac{10}{10000}$	0
"	"	"	$\frac{10}{10000}$	40	"	"	"	$\frac{5}{10000}$	0
"	"	"	$\frac{5}{10000}$	60	"	"	"	$\frac{3}{10000}$	0
"	"	"	$\frac{2.5}{10000}$	70	"	"	"	$\frac{1.5}{10000}$	0
"	"	"	$\frac{1}{10000}$	90	"	"	"	$\frac{2}{10000}$	3 (?)
					"	"	"	$\frac{1}{10000}$	0
					"	"	"	$\frac{1}{10000}$	0
					"	"	"	$\frac{1}{10000}$	0

should, according to Loeb's hypothesis, be equally well neutralized. This is, however, not the case. While there is no marked difference in the ease of neutralizing potassium and sodium chlorides, lithium chloride is much harder to neutralize, and ammonium chloride requires at least ten times as much calcium chloride for neutralization as does sodium chloride. This may be seen in Table IV.

The figures for lithium and sodium are given in Table I, and further experiments are cited for ammonium chloride later. As a $\frac{5}{8} m$ solution of ammonium chloride is not a fatal dose for some eggs, the difficulty of neutralizing by calcium is the more significant. The monochlorides arrange themselves in ease of neutralization in the order of their electrolytic decomposition tensions, this being highest in potassium chloride and lowest in ammonium chloride. The quantity of calcium chloride necessary to neutralize ammonium chloride is so much greater than that required to neutralize sodium chloride that little significance can be attached to the small quantity required in the former case.

If the anti-toxic action is a function of valence the polyvalent ions ought to be far more efficient than the bivalent. Loeb thought that aluminium was, as a matter of fact, more efficient than other ions; on

TABLE IV.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
KCl	$\frac{2}{8}$ M	5	KCl	$\frac{5}{8}$ M	CaCl ₂	$\frac{100}{8000}$	5
"	"	MgCl ₂	$\frac{100}{1600}$	5	"	"	"	$\frac{100}{4000}$	4
"	"	"	$\frac{100}{320}$	0	"	"	"	$\frac{100}{1600}$	20
"	"	"	$\frac{100}{160}$	12	NH ₄ Cl	$\frac{5}{8}$ M	0
"	"	"	$\frac{100}{100}$	22			MgCl ₂	$\frac{100}{333}$	0
"	"	"	$\frac{100}{80}$	30			"	$\frac{100}{160}$	0
"	"	"	$\frac{100}{28}$	30			"	$\frac{100}{80}$	0
KCl	$\frac{5}{8}$ M	10(?)			"	$\frac{100}{40}$	0
"	"	MgCl ₂	$\frac{100}{800}$	0			"	$\frac{100}{16}$	0
"	"	"	$\frac{100}{266}$	0	NH ₄ Cl	$\frac{5}{8}$ M	CaCl ₂	$\frac{100}{2000}$	0
"	"	"	$\frac{100}{200}$	10			"	$\frac{100}{1333}$	0
"	"	"	$\frac{100}{160}$	0			"	$\frac{100}{1000}$	0
"	"	"	$\frac{100}{80}$	10			"	$\frac{100}{800}$	20
"	"	"	$\frac{100}{40}$	14			"	$\frac{100}{400}$	35

the other hand, ferric chloride had no anti-toxic action.¹ My own results with aluminium chloride do not confirm the great activity of this salt, as may be seen in Table V. A solution of $\frac{5}{8}$ M NaNO₃ containing FeCl₃ in the concentrations $\frac{100}{300000}$, $\frac{100}{150000}$, $\frac{100}{100000}$, $\frac{100}{75000}$, $\frac{100}{60000}$, $\frac{100}{30000}$, $\frac{100}{20000}$, $\frac{100}{15000}$, $\frac{100}{10000}$, $\frac{100}{7500}$, $\frac{100}{5000}$, produced in no case a single embryo.

The minimum anti-toxic dose of aluminium chloride is therefore about an $\frac{100}{100000}$, as contrasted with an $\frac{100}{4000}$ of calcium chloride. It will be seen that this is a smaller difference than that between magnesium chloride and calcium chloride. In other words, there is a smaller difference in the efficiency of these bivalent and trivalent

¹ Cf. LILLIE: This journal, 1904, x, p. 419.

TABLE V.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
KCl	$\frac{1}{10}$ m	6	KCl	$\frac{1}{10}$ m	AlCl ₃	$\frac{1}{12500}$	6
"	"	AlCl ₃	$\frac{1}{100000}$	0	"	"	"	$\frac{1}{10000}$	20
"	"	"	$\frac{1}{33333}$	5	"	"	"	$\frac{1}{5000}$	26
"	"	"	$\frac{1}{20000}$	0	"	"	"	$\frac{1}{2000}$	22

cation salts than between the two bivalent salts. This certainly can hardly be interpreted as favorable to the valence hypothesis, although it may be that the relationships really existing are masked by the failure of some ions to penetrate the egg easily.

But one other combination remains to be tested, and that is the toxic and anti-toxic action of monovalent for monovalent, and bivalent for bivalent salts. That it is possible to neutralize salts by other salts of the same valence may be seen from Ringer's observations with sodium and potassium chlorides on the heart, and from my observations on motor nerves. Loeb himself states that the poisonous action of sodium chloride can be in part neutralized by potassium chloride, although heavy doses are required. Table VI gives the results.

TABLE VI.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
NaNO ₃	$\frac{5}{8}$ m	KCl	$\frac{1}{33}$	17
"	"	"	$\frac{1}{11}$	28
"	"	"	$\frac{1}{6.6}$	10

In other words, to neutralize sodium nitrate a concentration of potassium chloride less than $\frac{1}{33}$ is necessary. Contrast this with the amount of magnesium chloride required to give a similar number

of embryos. An $\frac{m}{100}$ magnesium chloride solution gave only 8 per cent of embryos. To get 17 per cent an $\frac{m}{90}$ solution must be used. In other words, it takes about twice as much potassium chloride to neutralize sodium chloride or nitrate as is required of magnesium chloride. This difference is immaterial when it is remembered that it takes twenty times as much magnesium chloride as calcium chloride. We find, as we did with aluminium, that there is no sharp difference between bivalent and monovalent cations in their neutralizing power, but that far greater differences exist between salts having ions of the same valence than between salts of ions of different valence.

With lithium chloride I obtained no neutralization of sodium chloride; but with hydrochloric acid the following result was obtained:

TABLE VII.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
KCl	$\frac{4.50}{1000}$	Control	..	0	KCl	$\frac{4.50}{1000}$	HCl	$\frac{12500}{m}$	4
"	"	HCl	$\frac{100000}{m}$	0	"	"	"	$\frac{10000}{m}$	4
"	"	"	$\frac{50000}{m}$	0	"	"	"	$\frac{5000}{m}$	10
"	"	"	$\frac{25000}{m}$	0	"	"	"	$\frac{3333}{m}$	40

There is here an unmistakable anti-toxic effect although it is slight except in the last concentration. This is nearly the minimum fatal dose for this acid, and the embryos barely reached the form of a line on the egg before dying. With potassium hydrate, on the other hand, no anti-toxic action was obtained.

The anti-toxic action of bivalent metals upon each other was also tested with positive results (Table VIII).

The experiments in Table VIII show very clearly that calcium chloride can neutralize the toxic action of magnesium chloride and of manganese. The anti-toxic power of the calcium salt for magnesium chloride is indeed greater than the anti-toxic action of strontium chloride upon sodium salts. Thus, magnesium chloride gave embryos with calcium chloride present in an $\frac{m}{800}$ concentration, while sodium chloride required an $\frac{m}{500}$ solution of strontium chloride.

TABLE VIII.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.	Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Per cent of embryos developed.
MgCl ₂	$\frac{5}{16} m$	0	MnCl ₂	$\frac{m}{10}$	0
"	"	CaCl ₂	$\frac{m}{3000}$	0	"	"	CaCl ₂	$\frac{m}{840}$	7
"	"	"	$\frac{m}{100}$	4	"	"	"	$\frac{m}{120}$	30
"	"	"	$\frac{m}{160}$	35	"	"	"	$\frac{m}{115}$	45
					"	"	"	$\frac{m}{180}$	40
					"	"	"	$\frac{m}{128}$	56
MnCl ₂	$\frac{m}{8}$	0					
"	"	CaCl ₂	$\frac{m}{840}$	2	MnCl ₂	$\frac{m}{15}$	30
"	"	"	$\frac{m}{120}$	8	"	"	CaCl ₂	$\frac{m}{840}$	30
"	"	"	$\frac{m}{115}$	11	"	"	"	$\frac{m}{115}$	60
"	"	"	$\frac{m}{160}$	8	"	"	"	$\frac{m}{128}$	78
"	"	"	$\frac{m}{128}$	25					

To neutralize manganese chloride, more calcium is necessary; but it will be seen that an $\frac{m}{320}$ solution gives 30 per cent of embryos in an $\frac{m}{10}$ manganese chloride solution. A quantitative difference, therefore, between the amounts of bivalent salts necessary to neutralize univalent, and the amounts necessary to neutralize bivalent does not exist. How can this anti-toxic action upon each other of salts of which the metals have the same valence be explained upon the valence hypothesis?

TABLE IX.

Salt.	Concentration.	Eggs.	Embryos developed.	Salt.	Concentration.	Eggs.	Embryos developed.
Co(NO ₃) ₂	$\frac{m}{1000}$	20	11	Co(NO ₃) ₂	$\frac{m}{165}$	19	7
"	$\frac{m}{800}$	33	9	"	$\frac{m}{125}$	30	5
"	$\frac{m}{115}$	19	3	"	$\frac{m}{100}$	26	0
"	$\frac{m}{250}$	34	4	"	$\frac{m}{50}$	20	1

I found it even possible to neutralize cobalt and nickel salts with calcium, and as these experiments are interesting for two reasons, I will give them in full (Tables IX and X).

TABLE X.

	Salt.	Concentration.	Eggs.	Embryos developed.
100 c.c.	$\text{Co}(\text{NO}_3)_2$	$\frac{m}{1000} + 2 \text{ c.c. } \text{CaCl}_2 \frac{m}{3}$	36	12
"	"	$\frac{m}{500} +$ " "	25	10
"	"	$\frac{m}{333} +$ " "	35	10
"	"	$\frac{m}{250} +$ " "	35	21
"	"	$\frac{m}{188} +$ " "	35	17
"	"	$\frac{m}{125} +$ " "	39	20
"	"	$\frac{m}{100} +$ " "	23	10
"	"	$\frac{m}{50} +$ " "	27	12
"	"	$\frac{m}{25} +$ " "	29	13

None of the embryos in the first six mixtures of the calcium and cobalt were coagulated, whereas without the calcium the cobalt soon coagulates. There is no mistaking, I think, the protective action of the calcium chloride.

The following experiment with nickel chloride will serve to show the peculiar action of this metal (Table XI).

TABLE XI.

Salt.	Concentration.	Eggs.	Embryos developed.	Salt.	Concentration.	Eggs.	Embryos developed.
NiCl_2	$\frac{m}{1000}$	18	0	NiCl_2	$\frac{m}{125}$	45	4
"	$\frac{m}{500}$	34	7	"	$\frac{m}{100}$	22	5
"	$\frac{m}{333}$	36	7	"	$\frac{m}{88}$	37	1
"	$\frac{m}{250}$	23	1	"	$\frac{m}{50}$	27	5
"	$\frac{m}{188}$	35	4				

This experiment shows that while nickel chloride in great dilution will kill nearly the whole of the eggs, a few eggs will resist enor-

mously greater doses. The figures show no increase in poisonous action in passing from an $\frac{m}{1000}$ to an $\frac{m}{50}$ solution. Beyond this all eggs are killed. This result is of interest for the reason that nickel and cobalt were marked exceptions to the rule of the relation of poison action and decomposition tension. According to the formula given in my paper, nickel chloride ought to be fatal in concentrations far below those found. This experiment indicates, I think, that the minimum fatal dose for this salt ought to be very close to its calculated value and far below that I assigned to it. For some reason, nickel chloride enters the egg with great difficulty. A few eggs hence escape its action, and these few resist enormous quantities and make the minimum fatal dose appear higher than it ought.

The action of calcium chloride on the poisonous power of nickel chloride is shown in the following experiment (Table XII):

TABLE XII.

Salt.	Concentration.		Eggs.	Embryos developed.
NiCl ₂	$\frac{m}{500}$	100 c.c.	19	4
"	"	100 c.c. + 2 c.c. CaCl ₂ $\frac{m}{8}$	38	35
"	$\frac{m}{250}$	100 c.c.	25	0
"	"	100 c.c. + 2 c.c. CaCl ₂ $\frac{m}{8}$	39	35
"	$\frac{m}{166}$	100 c.c.	34	0
"	"	100 c.c. + 2 c.c. CaCl ₂ $\frac{m}{8}$	25	18
"	$\frac{m}{125}$	100 c.c.	28	0
"	"	100 c.c. + 2 c.c. CaCl ₂ $\frac{m}{8}$	38	27
"	$\frac{m}{100}$	100 c.c.	20	0
"	"	100 c.c. + 2 c.c. CaCl ₂ $\frac{m}{8}$	43	31
"	$\frac{m}{50}$	100 c.c.	22	2
"	"	100 c.c. + 2 c.c. CaCl ₂ $\frac{m}{8}$	44	29

The anti-toxic action of calcium for nickel chloride is thus shown to be very great. A fatal dose of nickel chloride is no longer poisonous in the presence of $\frac{m}{50}$ calcium chloride in which nearly 90 per cent of the eggs develop into embryos, and many of them hatch.

The next experiment shows how small were the quantities of calcium chloride required (Table XIII).

TABLE XIII.

	Salt.	Concentration.	Eggs.	Embryos developed.
100 c.c.	$\text{Co}(\text{NO}_3)_2$	$\frac{m}{1000}$	29	11
"	"	" + 0.2 c.c. $\text{CaCl}_2 \frac{5}{8} m$	42	28
"	"	" + 0.4 " "	60	34
"	"	" + 0.6 " "	48	30
"	"	" + 0.8 " "	22	16
"	"	" + 1.0 " "	41	19
"	"	" + 1.5 " "	28	19

A plain effect can be seen with the calcium chloride present in $\frac{m}{800}$ concentration, and its action is therefore comparable to that of strontium upon sodium chlorides.

Is there a definite numerical relationship between the toxic action of a given number of molecules of one salt and one molecule of the anti-toxic salt?

It will be remembered that Hardy found a numerical relationship between the precipitating powers of mono- and bivalent cations. A bivalent cation was many times as powerful as a monovalent cation. Loeb¹ concluded that a somewhat similar relationship existed for the toxic and anti-toxic action. To neutralize a single molecule of potassium chloride a very small fraction of a molecule of calcium chloride was necessary, whereas to neutralize the poisonous action of a bivalent cation salt greater quantities were required. This numerical relationship, however, was determined for a single concentration, *i. e.*, $\frac{5}{8} n$ or $\frac{6}{8} n$ of the toxic univalent salt. It was desirable to see whether any such relationship actually existed.

If one molecule of calcium chloride would neutralize 1000 of potassium chloride in $\frac{5}{8} n$ solution, it ought to be possible by greater concentrations of calcium chloride to neutralize the poisonous power of more concentrated solutions of potassium chloride. I tried experi-

¹ LOEB: Archiv für die gesammte Physiologie, 1901, lxxxviii, p. 72; *ibid.*, p. 78, ¶ c.

ments to see how concentrated solutions of the different monovalent salts it was possible to neutralize with calcium chloride. The results showed a great variation in different salts (Table XIV).

TABLE XIV.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Molecular relationship.		Per cent of embryos developed.
				Toxin.	Anti-toxin.	
KCl	$\frac{3}{8}$	CaCl ₂	$\frac{100}{1000}$	1200	1	20
"	$\frac{6}{10}$	"	$\frac{100}{800}$	480	1	100
"	$\frac{7}{10}$	"	"	560	1	58
"	$\frac{8}{10}$	"	"	640	1	25
"	$\frac{9}{10}$	"	"	720	1	0
"	"	"	$\frac{100}{400}$	360	1	0
"	"	"	$\frac{100}{200}$	180	1	0
"	"	"	$\frac{100}{133}$	119	1	10
"	"	"	$\frac{100}{108}$	97	1	33
"	"	"	$\frac{100}{800}$	800	1	0
"	1.1 <i>N</i>	"	"	880	1	0
"	1.2 <i>N</i>	"	"	960	1	0

The foregoing experiment illustrates the fact that the molecular relationships of the toxin to the anti-toxin are not fixed. When potassium chloride is present in a $\frac{3}{8}$ *N* solution, it takes but one molecule of calcium chloride to 1200 of the potassium to yield 20 per cent of embryos. If, however, an $\frac{8}{10}$ solution is used, one molecule of calcium chloride to 640 of the potassium yields only 25 per cent of embryos. In a $\frac{9}{10}$ solution, the concentration of the calcium must be increased so that only one hundred and nineteen molecules of potassium chloride are present to one of calcium chloride in order to give any embryos. Above a $\frac{9}{10}$ normal solution I have not obtained any embryos, although greater concentrations of calcium chloride than I have tried might possibly yield a few.

With sodium acetate the following results were obtained, Table XV:

TABLE XV.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Molecular relationship.		Per cent of embryos developed.
				Toxin.	Anti-toxin.	
$\text{NaC}_2\text{H}_3\text{O}_2$	$\frac{1}{8} n$	CaCl_2	$\frac{m}{1600}$	5000.0	1	0
"	"	"	$\frac{m}{4000}$	2500.0	1	6
"	"	"	$\frac{m}{2666}$	1665.0	1	30
"	"	"	$\frac{m}{2000}$	1250.0	1	62
"	"	"	$\frac{m}{1600}$	1000.0	1	91
"	"	"	$\frac{m}{1066}$	665.0	1	100
"	$\frac{9}{16}$	"	$\frac{m}{213}$	119.0	1	100
"	$\frac{9}{14}$	"	$\frac{m}{187}$	117.0	1	100
"	$\frac{9}{12}$	"	$\frac{m}{140}$	180.0	1	0
"	$\frac{9}{10}$	"	$\frac{m}{266}$	239.0	1	0
"	$\frac{9}{10}$	$\text{Ca}(\text{NO}_3)_2$	$\frac{m}{43}$	38.7	1	90

It may be seen in this table that while sodium acetate in $\frac{5}{8} n$ solution is easily neutralized by very small quantities of calcium chloride, *i. e.*, one molecule to sixteen hundred of the acetate, in the slightly greater concentration of $\frac{9}{12}$ normal, a concentration of calcium ten times as great is wholly unable to yield a single embryo.

With ammonium chloride still more striking results were obtained. In a $\frac{5}{8} m$ solution of this salt some eggs developed. Even in a $\frac{6}{8} m$ solution an occasional egg may form an embryo. Yet it is possible only with large quantities of calcium to neutralize a $\frac{5}{8} m$ solution, as may be seen in Table IV. In a $\frac{6}{8} n$ ammonium chloride solution, still larger concentrations of calcium are required. No embryos were obtained with calcium chloride present in $\frac{m}{128}$ and $\frac{m}{64}$ concentrations. In an $\frac{m}{21}$ solution I obtained 13 per cent of embryos. This is a molecular relationship of 1 mol. calcium chloride to 16 of ammonium chloride.

With lithium chloride the result was obtained that while this salt may be easily neutralized in concentrations less than $\frac{3}{8}$ molecular, above a $\frac{1}{4}$ molecular concentration no amount of calcium chloride would give any embryos. This is shown in the following table (Table XVI):

TABLE XVI.

Toxic salt.	Concentration.	Anti-toxic salt.	Concentration.	Molecular relationship.		Per cent of embryos developed.
				Toxin.	Anti-toxin.	
LiCl	$\frac{3}{8}$	Ca(NO ₃) ₂	$\frac{110}{640}$	240.0	1	40
"	"	"	$\frac{110}{320}$	120.0	1	100
"	"	"	$\frac{110}{128}$	48.0	1	99
"	$\frac{1}{8}$	"	$\frac{110}{640}$	320.0	1	0
"	"	"	$\frac{110}{213}$	106.0	1	0
"	"	"	$\frac{110}{64}$	32.0	1	11
"	"	"	$\frac{110}{27}$	13.0	1	23
"	$\frac{5}{8}$	"	$\frac{110}{320}$	200.0	1	0
"	"	"	$\frac{110}{160}$	100.0	1	0
"	"	"	$\frac{110}{64}$	40.0	1	0
"	"	"	$\frac{110}{27}$	17.0	1	0
"	$\frac{3}{8}$	"	$\frac{110}{64}$	36.0	1	0
"	"	"	$\frac{110}{32}$	18.0	1	0
"	"	"	$\frac{110}{21}$	15.7	1	0

While, therefore, in a $\frac{3}{8} n$ lithium chloride solution, a molecule of calcium chloride to each 120 of lithium chloride will yield 100 per cent of embryos, in a $\frac{1}{8} n$ solution, one molecule of calcium chloride to 106 of lithium chloride yields nothing, and 1 to 32 lithium chloride gives only 11 per cent.

These figures show that a definite molecular relationship between the toxin and anti-toxin does not exist, and the conclusion is not justified that one molecule of a bivalent cation salt is anti-toxic for a very large number of molecules of the monovalent toxic salt. This relationship is true only for certain concentrations of sodium and potassium salts. Lithium and ammonium salts require quite different molecular amounts of calcium from these other salts.

The conclusions from these experiments may be summarized as follows:

1. The toxic action of any salt shows no definite relationship to the valence of either ion, but is related to the decomposition tension of the salt.

2. The toxic action is due to both ions equally. The anti-toxic action must hence involve both ions.

3. The anti-toxic action of the salts of the bivalent metals for the salts of the univalent metals involves all ions both of the anti-toxin and the toxin, and is not due to an anti-toxic action between different cations of different valence.

4. The salts of different bivalent metals show the widest variations in anti-toxic action, ranging from no anti-toxic action at all, to very strong anti-toxic action. Magnesium chloride is only $\frac{1}{20}$ as strong as calcium chloride.

5. It is possible to neutralize the toxic action of monovalent by monovalent salts and bivalent by bivalent salts.

6. The amount of calcium chloride or other bivalent salt required to neutralize different monovalent salts is different for every salt. Ammonium and lithium chlorides are neutralized with the greatest difficulty.

7. The number of molecules of calcium chloride required to neutralize one molecule of any monovalent salt is not a fixed quantity, but varies with the concentration of the monovalent salt. In some cases a very slight increase in the concentration of the monovalent salt makes it impossible to neutralize its toxic action except by enormous doses of calcium chloride. No characteristic quantitative relationship between monovalent and bivalent salts in their anti-toxic relationships is to be discerned.

8. *From these facts it is clear that valence, as such, either of the anion or the cation is of secondary or no importance in determining either the toxic or anti-toxic action of the salts.*

So far the conclusions reached have been negative. What is then the true explanation of anti-toxic action? I am not able to answer this question as a whole, but the following considerations explain some of the phenomena.

In the first place, is there any relation between the decomposition tension of the salt and its anti-toxic power?¹ The facts show that only those salts of sodium, potassium, and lithium of a high decomposition tension can be neutralized. As soon as an anion of low

¹ LILLIE (This journal, 1904, x, p. 443) finds such a relationship for the anti-toxic action of ions on ciliary movement.

solution tension is added, it becomes much more difficult or altogether impossible to neutralize the compound. In the second place, the anti-toxic metals all fall practically in the list of solution tensions above cobalt. They are metals for the most part of high decomposition tension. Whether, however, this relationship can be extended farther than this general statement, and an explanation of the facts be founded upon it, cannot at present be said.

In the second place, a part of the anti-toxic action is undoubtedly due, I think, to an alteration in permeability of the egg membranes by the anti-toxic salt. The blood and presumably the tissue of the teleost fishes have an osmotic pressure of about one-half that of the sea-water. The monovalent salts which can be counteracted by calcium and other salts are only poisonous in concentrations approximately equal to, or in some cases in excess of the osmotic pressure of the sea-water. When a *Fundulus* egg is brought into a sodium chloride solution of $\frac{5}{8}$ *n* concentration a difference of about twelve atmospheres of pressure must exist between the inside and the outside of the egg. It is clear that were the egg membranes not readily permeable to salts, this great pressure would at once plasmolyse the egg.¹ A very slight change in permeability could, therefore, profoundly effect the ease of plasmolysis. There is evidence that the poisonous action of sodium, ammonium, lithium, and potassium chlorides is due in part to the fact that they cannot enter the egg with entire freedom. When put into such solutions, the protoplasmic envelope of the egg may be seen to shrink away from the outer membrane, and in case the solution is strong enough, complete plasmolysis takes place and the membrane is driven into a compact mass at the centre of the egg. A part of the poisonous action is, therefore, undoubtedly due, I think, to the plasmolysing action of the salts. It is this plasmolysing action particularly which calcium salts and other bivalent metals are able to offset. If the eggs are placed in $\frac{9}{8}$ – $\frac{7}{8}$ *n* potassium chloride solutions containing calcium or any other anti-toxic salt, this plasmolysis does not occur. The egg does not shrink, but remains uncoagulated and of its normal size.

These facts point to the conclusion that a part, at least, of the anti-toxic action is due to an alteration in permeability of the cell-membrane by the calcium or other anti-toxic salt, as suggested by

¹ LOEB refers to *Fundulus* eggs as being less permeable to salts than *Arbacia*. The contrary is the case, as otherwise *Fundulus* would be plasmolysed more easily than *Arbacia*.

Stewart.¹ This alteration is of such a sort that there is an increased permeability for lithium, sodium, potassium, and ammonium compounds. By this means, that part of the poisonous action of the salt which is due to plasmolysis is neutralized. Whether this explains the whole of the anti-toxic action is at present uncertain. It may be that calcium may also neutralize the specific poisonous action of the salts, but this appears to me doubtful. This explanation clears up also the fact that while potassium chloride may be neutralized up to a 0.9 *n* solution, lithium chloride can hardly be neutralized in concentrations beyond its minimum fatal dose. The reason for this would be that the specific poisonous power of lithium chloride, as computed from its decomposition tension, is almost that of its minimum fatal dose. That is, only a small part of the poisonous action of a $\frac{3}{8}$ *n* lithium chloride solution is due to plasmolysis; it is, therefore, impossible to neutralize lithium in much greater concentrations. With potassium, however, much of its poisonous action is due to plasmolysis. Its true poisonous dose should be 0.9 *n*. It is possible to neutralize potassium chloride in concentrations weaker than this, but not above it.

This explanation can obviously not apply to such salts as manganese chloride, cobalt chloride, and nickel chloride, which can also be neutralized by calcium chloride, but which are poisonous in concentrations far lower than that necessary to plasmolyse. The explanation of this antagonism, in my opinion, is that calcium *reduces* the permeability of the egg membranes to cobalt and nickel and manganese salts. The evidence for this view is as follows:

Normally, cobalt and nickel find their way into the egg with great difficulty. This is shown by the fact that the action of these salts is extremely irregular. Whereas the great majority of the eggs exposed to their action are killed by concentrations less than $\frac{m}{1000}$, a certain number of the eggs withstand concentrations as high as $\frac{m}{50}$. Furthermore, when these salts enter the egg, they produce a quite different change from that produced by poisonous doses of sodium chloride. In the former case there is no plasmolysis, but instead the embryo turns white or pink and is coagulated *in situ*. The action of the salts is also very slow, as if they entered with difficulty. While many embryos may live in an $\frac{m}{500}$ solution for twenty-four hours, most

¹ STEWART: American year book of medicine and surgery (medicine), 1904, p. 527; Cf. This journal, 1903, ix, p. 96; Comptes rendus, xiv, Congrès international de médecine, 1903, p. 84, Section de physiologie.

of these are coagulated before forty-eight hours. The same appearances are true for manganese, though the embryos are not so easily coagulated by this salt.

The appearance of the embryo when developing in the presence of a mixture of calcium and cobalt or nickel salts is quite different from that in cobalt or nickel without the calcium. In the latter case most eggs are coagulated and white; in the former they do not coagulate. We may conclude, therefore, that when calcium is present, the cobalt, nickel, and manganese do not enter the egg, and accordingly the embryo is protected from them.

How the permeability is altered by calcium is not clear. It may be, however, that the membrane is slightly coagulated, so that its texture is coarser, and potassium, ammonium, and sodium salts can pass through more readily. As regards cobalt and nickel, these ions may have a bulk too great to pass through even the coarser membrane. They may find their way into the egg ordinarily by first combining with the membrane, and in this way getting in.¹ If calcium is already in combination they can no longer combine with the membrane and they are too large to enter the free spaces in the membrane. Whatever the exact mechanism by which a change in the permeability is produced may be, I feel confident that a portion at least of the anti-toxic action is to be explained by this alteration in permeability of the egg membranes.

Further evidence of this alteration in permeability was secured in the following way: Sea-urchin eggs will not divide after fertilization if the concentration of the sea-water is raised. The careful examination of the segmentation problem by Spaulding² led him to conclude that this process involved a balance between intrinsic and extrinsic forces. Osmotic pressure is one of the forces which tends to check cell-division, since it tends to drive the egg into a spherical form and to prevent any enlargement of its surface. Now if a sea-urchin egg is placed in pure sodium chloride of an osmotic pressure equal to that of the sea-water, it shrinks, becomes spherical and will not divide, or divides very slowly. This shows that in sodium chloride, although the actual osmotic pressure is the same as that of sea-water, its efficiency is increased. This increased efficiency indicates that the egg membranes are more permeable to sodium chloride in

¹ It is possible that the bulk of the free ion of cobalt is very much greater than the atom in a non-ionized form.

² SPAULDING: Biological bulletin, 1904, vi, p. 98.

sea-water than to sodium chloride alone. In other words, the resistance of the egg membrane to the passage of sodium chloride is greater in pure solutions of this salt than when other salts are present. If this is the case, the addition of calcium chloride may be expected to reduce the effect of the sodium chloride and to permit the egg to segment. Experiment confirms this hypothesis, at least in part, as is shown by Loeb's observations on the effect of calcium on eggs in sodium chloride solutions. The following experiment was tried to test this hypothesis further (Table XVII).

TABLE XVII.

Arbacia eggs fertilized at 10.45 A. M., and transferred to the solutions noted.
Examined at 2 P. M.

100 c.c. NaCl.		100 c.c. NaCl + 0.7 c.c. $\frac{5}{8}$ m CaCl ₂ .	
Concentration.	Result.	Concentration.	Result.
1. $\frac{3}{8}$ n	Most are dead in 2-16 cells	1. $\frac{3}{8}$ n + CaCl ₂	Most alive; 2-32 cells.
2. $\frac{3}{8}$ n	In ten eggs there is one 5 cells; nine 2-32 cells	2. $\frac{3}{8}$ n + CaCl ₂	Twelve eggs, 16-20 cells; ten, 8 cells. Alive.
3. $\frac{4}{8}$ n	Eleven 1 cell; sixteen 8 cells	3. $\frac{4}{8}$ n + CaCl ₂	Eggs in 16-64 cells. Normal.
4. $\frac{4}{8}$ n	Majority undivided and dead	4. $\frac{4}{8}$ n + CaCl ₂	All 64 cells. Alive.
5. $\frac{5}{8}$ n	All dead	5. $\frac{5}{8}$ n + CaCl ₂	Alive. 2-32 cells.
6. $\frac{5}{8}$ n	Eggs black and shrunken	6. $\frac{5}{8}$ n + CaCl ₂	Undivided for the most part, but normal looking.

There are, however, certain differences in the reactions of these eggs from those of *Fundulus*. Thus calcium chloride would not neutralize the action of potassium chloride nearly as well as it did sodium. In fact I could get no neutralization of the poisonous action of potassium for these eggs, a fact which indicates that the anti-toxic action is not due in all likelihood to the same causes in different cases. Barium chloride also showed itself almost inert as an anti-toxic agent in all concentrations tried, but was itself exceedingly toxic. Magnesium chloride showed itself the best antagonist of sodium chloride.

The facts indicate an increase in permeability of the cell wall due to magnesium, calcium, and barium ions, but show clearly that there are certain other harmful actions of potassium and sodium compounds which these ions cannot inhibit.

The general conclusion to be drawn from these experiments is that the interpretation of the anti-toxic action of one salt upon another given by Loeb is incorrect, at least in part. Valence either of the cation or anion appears to have no share either in toxic or anti-toxic action. While this part of Loeb's explanation is wrong, it may still be that the action involves the electrical charges upon the ions, but in the way stated in my papers on solution tension and nerve stimulation.¹ It appears probable that several different causes may operate in determining anti-toxic action. In some cases the anti-toxic salt increases the permeability of the cell-membranes, thus relieving the cell from plasmolysis injuries; in other cases the cell-membrane is rendered less permeable to a given ion, thus protecting the protoplasm within from its influence. A part of the action may, however, be due to the necessity of preserving a certain viscosity of the protoplasm, in order that delicately adjusted protoplasmic processes may go on, and for this viscosity ions of definite potential must be present in proper proportions. It is probable, I think, that the facts showing the advantage of three or more metals for ciliary and muscular movement, and possibly nuclear division, are to be thus explained. In so complicated a process as cell-division, in which surface tension, protoplasmic viscosity, and respiration play a part, it is probable that all these factors come into play, and that while the anti-toxic action of calcium upon sodium chloride may be pronounced in its effects, for example, on surface tension, the salts may not antagonize each other's harmful action in their relations to other processes, such as karyokinesis.²

If the valence of an ion is of little or no importance in determining its anti-toxic action, attention must be directed to its other properties. Among these the ionic potential is indicated, both by Lillie's results and mine, to be of great importance, but it is not impossible that the ionic weight, velocity, and volume will all be found to play a part.

¹ MATHEWS: This journal, 1904, xi, p. 455.

² This general conclusion is practically the same as that reached by STEWART: American year book of medicine and surgery (medicine), 1904, p. 527.

THE QUANTITATIVE ESTIMATION OF CARBAMATES.

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THE physiological significance of carbamates was first pointed out by Drechsel.² This worker found carbamates to be formed when amido acids and other nitrogenous organic compounds were oxidized in the presence of alkalies, and he was also able to demonstrate traces of them normally present in the blood serum of dogs. Hahn and Nencki³ further found carbamates present in minute traces in the normal acid urines of the dog and man. In the alkaline urine of horses very considerable amounts of carbamates have been detected by Drechsel and Abel,⁴ and, by feeding dogs with lime until the urine became alkaline towards litmus, Abel and Muirhead⁵ were able to separate, in a comparatively pure state, considerable quantities of this substance.

In a series of dogs in which an anastomosis of the portal vein with the inferior vena cava had been successfully established by Pawlow and Massen, Hahn and Nencki demonstrated considerable quantities of carbamate in the urine, and these workers concluded that the nervous symptoms which frequently make their appearance after this operation are due to the presence of excess of carbamates in the blood.⁶ The amount of carbamates was found to be still greater when, in the Eck's fistula dogs, the liver was also removed from the circulation by ligation of the hepatic arteries. Lieblein⁷ has also noticed

¹ H. M. Hanna Research Fellow, Western Reserve University.

² DRECHSEL: *Vide* NOLF: *Zeitschrift für physiologische Chemie*, 1897, xxiii, p. 505.

³ HAHN and NENCKI: *Archiv für experimentelle Pathologie und Pharmakologie*, 1893, xxxii, p. 185.

⁴ DRECHSEL and ABEL: *Archiv für Physiologie*, 1891, p. 231.

⁵ ABEL and MUIRHEAD: *Archiv für experimentelle Pathologie und Pharmakologie*, 1892, xxxi, p. 15.

⁶ PAWLOW, MASSEN, HAHN, und NENCKI: *Archiv für experimentelle Pathologie und Pharmakologie*, 1893, xxxii, p. 161.

⁷ LIEBLEIN: *Archiv für experimentelle Pathologie und Pharmakologie*, 1894, xxxiii, p. 327.

the presence of an excess of carbamate in the urine of dogs after partial destruction of the hepatic cells by injecting dilute sulphuric acid into the biliary ducts.

The chemical method mainly employed in the above investigations for the detection of carbamates was that recommended by Drechsel.¹ This method consists in shaking the urine for ten minutes with an excess of milk of lime; the solution is then filtered or centrifugalized, and the clear fluid, thus obtained, again shaken with a few drops of a solution of calcium chloride and with a few crystals of calcium carbonate. By this second precipitation, all traces of soluble carbonate are converted into the calcium salt which, by the shaking with crystalline carbonate, is rendered crystalline, and therefore easily separable by filtration. By the above process any carbamates which the solution may contain are converted into the calcium salt, which is soluble in water. After standing on ice for some time, the solution is filtered into an excess of alcohol at a temperature of 0° C., in which — after again standing for some time — calcium carbamate separates out as a precipitate, which, however, is highly impure. To purify it, the precipitate is dried in a desiccator, and then dissolved in a small amount of 10 per cent ammonia, the resulting solution being then subjected to fractional precipitation, the third precipitate obtained being comparatively pure carbamate. This final precipitate, when dissolved in ice-cold water and the resulting solution placed in a closed tube, gives a solution which is at first perfectly clear, but which, on raising the temperature, becomes cloudy from the separation of calcium carbonate.

The preparation thus obtained is, however, far from pure;² for, when it is dissolved in water and the resulting solution heated, a very variable amount of ammonia and carbon dioxide gas is obtained.³ Theoretically, for each molecule of carbon dioxide which such a solution yields, there ought to be two molecules of ammonia, as is evident from the following equation:



The above method is entirely unsuited for quantitative work.³

¹ DRECHSEL and ABEL: *Loc. cit.*, p. 238.

² Aromatic sulphates and volatile fatty acids were found by HAHN and NENCKI (*loc. cit.*) in the preparation.

³ NEUBAUER and VOGEL: *Analyse des Harns*; analytischer Theil, 1898, p. 270.

It has been further pointed out, especially by Nolf¹ and Hofmeister,² that during the manipulations of Drechsel's method a considerable amount of carbamate may be formed. These authors point out that any watery solution of ammonium carbonate must contain at least some carbamate, and that when such solution is shaken with milk of lime, the amount of carbamate will increase, on account of the liberation of free ammonia. Not only ammonium carbonate, but any carbonate, will form carbamate when shaken with alkalies in the presence of substances which readily yield ammonia (Hofmeister), or where free carbon dioxide gas exists along with ammonia salts.

In all these cases the formation of carbamates can be explained by the chemical interaction between carbonates and ammonia, so that much doubt is thrown on the existence of preformed carbamates in animal fluids.

The importance of a reliable test for the presence of carbamates, and the possibility of securing a method for estimating the amount of them present in animal fluids, especially in conditions where the circulation through the liver is interfered with, as after an Eck's fistula has been instituted, prompted us to see whether, by an estimation of the carbon dioxide gas in fluids, before and after precipitation of the carbonates by suitable reagents, a more reliable method than that of Drechsel could not be obtained. In this account we will confine ourselves to a description of the method as used for various fluids, and to proofs of its accuracy; and we will reserve for a future paper a fuller account of the results of bio-chemical interest which have been obtained by it.

The principle of the method is as follows: the carbon dioxide gas in 1 c.c. of the fluid under examination is estimated in the apparatus of Barcroft and Haldane,³ the temperature of the water bath being noted on a delicate thermometer.

Another cubic centimetre of the fluid is vigorously shaken in a stoppered weighing bottle with an excess of a saturated watery solution of barium hydroxide⁴ containing ammonia. This causes complete precipitation of the carbonates, but not the carbamates, as the barium salt of these is soluble in water (Drechsel). After about one-

¹ NOLF: *Loc. cit.*

² HOFMEISTER: *Archiv für die gesammte Physiologie*, 1872, xii, p. 337.

³ BARCROFT and HALDANE: *The journal of physiology*, 1902, xxviii, p. 232.

⁴ For this shaking we have recently used a mechanical shaking apparatus driven by a water motor.

half hour the fluid is transferred to a tube, which is tightly corked and centrifuged, and the carbon dioxide gas determined (at the same temperature as that used for total carbon dioxide), either in the supernatant fluid or in the precipitate; in the supernatant fluid when blood, or other fluids rich in proteids, is under examination; in the precipitate in other cases. The gas found in the supernatant fluid corresponds to that of carbamates. The difference between the amount of gas found in one cubic centimetre of the total fluid, and that found in the precipitate, also corresponds to the gas of carbamates.

We will first consider *the method employed for simple solutions of carbamates, i. e.*, where the precipitate is examined. The reliability of this method must depend mainly on the entire precipitation of carbonates by barium hydroxide and ammonia. That such is the case is proved by the following results:

1. 0.5 c.c. $\frac{N}{16}$ sodium carbonate (approx.) gave, when mixed with tartaric acid in the Barcroft and Haldane apparatus, 0.584 c.c. carbon dioxide.

0.5 c.c. of the same solution was mixed with baryta water and ammonia, shaken and centrifuged. The precipitate washed into the gas bottle and mixed with tartaric acid gave:

- a. 0.581 c.c.
- b. 0.582 c.c.
- c. 0.571 c.c.

2. In another series of tests with 0.5 c.c. sodium carbonate solution which had stood for some time, 0.567 c.c. and 0.559 c.c. carbon dioxide were found, and the barium precipitate obtained as above described gave 0.557 c.c. and 0.566 c.c.

The presence of other neutral salts besides carbonates does not interfere with this precipitation.

1. 1 c.c. of an artificial plasma solution (made by dissolving the various salts of plasma in proper proportions in distilled water) gave 0.593 c.c. of carbon dioxide. The barium precipitate obtained by the above method gave 0.586 c.c.
2. 1 c.c. freshly passed human urine containing added sodium carbonate gave 0.566 c.c. and 0.552 c.c. carbon dioxide. The barium precipitates treated as above gave 0.566 c.c. and 0.578 c.c.

In the above cases, viz., where phosphates, etc., exist in the fluid, a heavy precipitate follows the addition of barium hydroxide, and, to entirely displace all the carbon dioxide gas from this precipitate, an

excess of tartaric acid must be added. This we accomplish by using, instead of the glass spoon supplied with the apparatus, a small flat bottomed tube, closed at one end, and holding about 1 c.c. of acid; this tube is placed in the gas bottle, and used in the same manner as that employed in decomposing urine with hypobromite.

The presence of proteids retards considerably the precipitation of the carbonates, but, with certain precautions, the entire precipitation of these can be effected. Without adopting these precautions, results like the following are obtained:

1. 1 c.c. of sodium carbonate solution containing egg proteid gave 0.680 and 0.699 c.c. gas. The barium precipitates from two samples of 1 c.c. each of this solution gave 0.561 c.c. and 0.593 c.c.
2. 1 c.c. of artificial plasma containing egg proteid gave 0.648 c.c. and 0.655 c.c. carbon dioxide. 1 c.c. of the same solution, precipitated as above with barium, and the precipitate thoroughly washed several times with weak ammonia water,—the supernatant fluid being removed by decantation,—gave 0.526 c.c. gas. With blood plasma even worse results are obtained.

If, however, the proteid solution be considerably diluted with weak ammonia, and be treated with excess of barium solution and frequently shaken for one and a half hours, the precipitation of carbonates is much more complete.

1. 1 c.c. 25 per cent egg white in artificial plasma solution gave 0.566 c.c. and 0.566 c.c. carbon dioxide. 1 c.c. of the same solution with 8 c.c. barium hydroxide solution and 10 c.c. ammonia water (0.5 per cent), after frequent shaking during 1½ hours, was centrifuged until the supernatant fluid was clear. The precipitate was then washed twice by decantation with 0.5 per cent ammonia, and yielded 0.753 c.c. carbon dioxide.

In this case it is obvious that carbonates had been formed, during the prolonged standing, from the carbon dioxide in air. To correct for this error, a blank of 8 c.c. barium hydroxide solution and 10 c.c. 0.5 per cent ammonia was carried out as above, and the precipitate gave 0.185 c.c. gas. Deducting this latter figure from that for the first precipitate, 0.568 c.c. carbon dioxide is obtained.

Exactly similar determinations were made with blood serum; the following table (No. I) gives the results:

TABLE I.

Fluid examined.	Total carbon dioxide.	Carbon dioxide in precipitate.	Carbon dioxide in blank.	Carbon dioxide of carbonates precipitated.
	c.c.	c.c.	c.c.	c.c.
Blood serum	0.380	0.544	0.158	0.386
Blood serum	0.377	0.535	0.158	0.377
Blood serum	0.382	0.333	0.074	0.259
Blood serum	0.370	0.301

From this table it is seen that, although by frequent shaking it is possible to precipitate all the carbonates, the method is an uncertain one and difficult.

This uncertainty is due to the difficulty in shaking down the carbonate precipitate. We will describe later a modification of the method which we use for blood serum.

To summarize, all the carbonate in simple solutions, or in solutions such as artificial plasma or urine which contain no proteid, can be precipitated by shaking with a saturated solution of barium hydroxide, containing 0.5 per cent ammonia; where, however, any considerable amount of proteid is present in such solutions the precipitation of carbonates is uncertain.

The second factor on which the reliability of the method depends, is that saturated baryta solution does not have any action on carbamates, that it does not accelerate the decomposition of carbamates into carbonates.

This can be easily shown by dividing a solution of calcium carbamate in ice-cold water into two equal parts, and adding barium hydroxide to one of these. If kept cool in tightly corked tubes, both solutions remain clear for an equal period of time.

The table on the following page (No. II) depicts some of the results which we have obtained by the above method in simple solutions of carbamates.

We give these results for the purpose of demonstrating the utility of the method, and we do not intend, in this article, to discuss in any detail the facts relating to the behavior of carbamate solutions which they demonstrate. It may be stated, however, that the results are entirely confirmatory of what is already known regarding this subject, viz., that the stability of such solutions depends on temperature

(1 and 2, 3 and 4), tension of ammonia present (9, 10, 11, and 12), and exposure to air (2 and 3). By exposure to air the ammonia formed by decomposition of the carbamate diffuses out of the solution, so that all the carbamates soon become decomposed; whereas,

TABLE II.

No.	I. Nature of solvent used.	II. Amount of carbamate of ammonium added.	III. Carbon dioxide gas in 1 c.c. of fluid.	IV. Condition under which solution was before baryta, etc., was added.	V. Length of time solution stood before baryta, etc., was added.	VI. ¹ Carbon dioxide gas in precipitate of 1 c.c. of fluid.	VII. Difference between III and IV, i. e., gas of carbamate.
		gram	c.c.			c.c.	c.c.
1	Distilled water	0.0016	0.476	In open bottle at room-temperature	$\frac{1}{4}$ hour	0.479	..
2	"	"	0.464	In open bottle on ice	1 hour	0.450	0.014
3	"	Not weighed	0.490	In closed flask on ice	$2\frac{1}{4}$ hours	0.422	0.068
4	"	"	0.408	In closed flask at body-temperature	20 min.	0.408	..
5	Lime water	"	0.493	In closed tube on ice	$1\frac{1}{2}$ hours	0.181	0.312
6	"	"	0.580	In closed flask at room-temperature	1 h. 20 m.	0.299	0.281
7	"	"	0.411	In closed flask at body-temperature	20 min.	0.218	0.193
8 ²	"	"	0.896	In loosely corked flask at room-temp.	2 days	0.972	..
9	1% ammonia	"	0.486	In closed tube on ice	$1\frac{1}{2}$ hours	0.218	0.268
10	0.33% ammonia	"	0.827	In closed flask at room-temperature	1 h. 10 m.	0.204	0.623
11	0.5% ammonia	"	0.196	In closed flask at body-temperature	20 min.	0.133	0.063
12 ³	0.33% ammonia	"	0.973	In loosely stoppered flask at room-temperature	2 days	1.085	..
¹ In none of these was any correction made for carbon dioxide absorbed from the air during the manipulations, nor was any great precaution taken to have the temperature of the water bath always the same. ² Excess of gas in VI due to absorption of CO ₂ from air. ³ Excess of gas in VI due to absorption of CO ₂ from air.							

when this diffusion is prevented, a solution of carbamate may remain undecomposed for days, even at room-temperature. The results further show that the calcium salt is more stable than the ammonia salts. (Compare 3 and 4 with 5, 6, and 7.)

We have also made several estimations by the above method of the

amount of carbamate in the urine of a dog fed on lime.¹ The following table gives the results, as well as results obtained by the same method on normal dog's urine:²

TABLE III.

I. No.	II. Amount of urine used for examination.	III. Carbon dioxide in II.	IV. Carbon dioxide in barium precipitate of II.	V. Carbon dioxide in barium, contrl.	VI. Carbon dioxide in carbonates of II. (= III - V).	VII. Carbon dioxide in carbonates of II. (= III - VI).	VIII. Carbon dioxide of carbamates per c.c.
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
1	3	0.189	0.1580	0.048	0.110	0.079	0.026
2	3	0.134	0.1211	0.033	0.088	0.046	0.015
3	1	1.022	0.9560	0.019	0.937	0.085	0.085
4	1	0.635	0.5460	0.020	0.526	0.109	0.109
5	1	1.934	1.7300	0.023	1.707	0.224	0.224
6	3	0.0335	0.0480
7	3	0.0737	0.0988	0.020	0.0788
Nos. 1 to 5 are from a lime-fed dog; Nos. 6 and 7 from a normal dog.							

The estimations confirm Abel and Muirhead's discovery³ that carbamates appear in the urine of dogs to which lime is administered until the urine reacts alkaline to litmus. Our results show further that the amount of carbamate steadily increases from day to day until the investigation has to be terminated on account of gastric disturbance. We found it essential for satisfactory results that only catheter specimens be examined. If the urine be collected in the ordinary metabolism cage, fermentation of urea is apt to set in, and ammonium carbonate to be formed, a certain amount of which becomes converted into carbamates.⁴ In one specimen of urine (No. 4), the carbamates were isolated by Abel and Drechsel's method. In all the estimations a control of the reagents in the same quantities

¹ Vide ABEL and MUIRHEAD: *Loc. cit.*

² Catheter specimens were used throughout, and were examined as soon after collection as possible.

³ ABEL and MUIRHEAD: *Loc. cit.*

⁴ NOLF: *Loc. cit.*

as used for precipitation was carried through, and the carbon dioxide gas found in the resulting barium carbonate precipitate—derived from air—deducted from that of the barium carbonate precipitate of the urine.

On account of the uncertainty of precipitating all carbonates by the above process *in solutions containing proteids*, we have found it necessary to adopt an entirely different method for estimating carbamates in such fluids. The principle of this latter method is similar to that used for non-proteid solutions, only that, instead of the precipitate, an aliquot part of the supernatant fluid is taken for estimation of carbon dioxide gas. (See page 447.)

Since in transferring this solution from the centrifuge tube to the blood-gas bottle of the Barcroft-Haldane apparatus, carbon dioxide is absorbed from the air by the barium hydroxide which the solution contains, it is necessary, in all estimations, to carry out controls, and to deduct from the amount of gas found in the solution under examination, the gas which is produced in the control.

On account of the readiness with which watery solutions of carbamates undergo decomposition into carbonates at room-temperature, we have found it necessary to insure the presence in the solution of a considerable tension of ammonia. This precaution is especially necessary when the percentage of carbamate is low. On the other hand, an excess of ammonia—in which, of course, decomposition of even larger amounts of carbamates would be entirely prevented—cannot be used, and for two reasons: firstly, because the ammonia would form carbamates with the carbonates present in the serum,¹ and secondly, because, with an excess of ammonia, the estimation of the gas in the Barcroft-Haldane apparatus is rendered inaccurate.

Before proceeding further with the elaboration of the method, therefore, it was necessary for us to determine what percentage of ammonia might be used without creating either of the two errors indicated above. For this purpose we have carried out several tests of which the following is a type:

Into five small test-tubes were placed, respectively, 2 c.c. water; 2 c.c. 0.5 per cent ammonia;² 2 c.c. 1 per cent ammonia; 2 c.c. 2.5 per cent ammonia; and 2 c.c. 5 per cent ammonia. In each of

¹ Vide DRECHSEL, HOFMEISTER, NOLF: *Loc. cit.*

² Throughout the paper these figures represent per cent of *aqua ammonia*. Specific gravity, 0.9.

the solutions 0.001 gr. calcium carbamate¹ was dissolved, and the tubes corked and left standing at room-temperature. In about one minute the watery solution had become densely opaque from the separation of calcium carbonate. In a few minutes calcium carbonate had also commenced to separate from the 0.5 per cent solution, and a little later from the 1 per cent solution; but the 2.5 per cent solution was perfectly clear, even after standing one hour. A tension of 2.5 per cent ammonia, therefore, prevents the decomposition of 1 mg. calcium carbamate. With regard to the second precaution, interference with the accuracy of the gas-determination in the Barcroft-Haldane apparatus, a 5 per cent solution of ammonia gives a dense precipitate with tartaric acid, and this interferes with the reading. With a 2.5 per cent solution, however, only a few crystals separate out and the reading is accurate.

The more carbamate present, the higher must be the tension of ammonia to prevent decomposition, and the choice of 1 mg. for the above tests may seem somewhat arbitrary; but 1 c.c. of blood serum could seldom be expected to contain more carbamate than 1 mg., for this amount of calcium carbamate itself gives 0.262 c.c. of carbon dioxide gas, and 1 c.c. of blood under normal conditions from 0.320-0.400 c.c. Even should the blood serum contain considerably more carbamate than this, it is improbable that any decomposition into carbonate would occur within the hour which is required for precipitation and centrifugalization.

The presence of such considerable amounts of ammonia introduces other difficulties besides the formation of a precipitate when the fluid is transferred to the gas bottle, viz., that it takes a large amount of the tartaric acid solution to neutralize all the ammonia, and that when the solution is shaken in the bottle, a minus reading, due to suction pressure, may result. This suction pressure persists after the fluid in the blood-gas bottle has been rendered distinctly acid, and its cause we cannot explain. Both these difficulties can be removed by nearly neutralizing the fluid before connecting up the blood-gas bottle with the manometer, and completing the acidifying after the manometer has been connected by adding 0.25 c.c. of tartaric acid previously placed in the spoon attached to the stopper of the apparatus.

To insure complete precipitation of carbonates, we also add, besides the above reagents, a small amount of barium chloride solution.

¹ As nearly as could be weighed.

The method for blood and similar fluids is briefly as follows: The blood is collected directly from the blood-vessel in a clean, dry centrifuge tube of 12–16 c.c. capacity, and containing a little mercury. The tube is tightly corked and then vigorously shaken. By this process the blood is defibrinated. The tube is then placed on the centrifuge. While the blood is being centrifuged, a mixture of 7 c.c. of clear baryta solution, 2 c.c. water previously boiled to remove all gas, and 0.5 c.c. of a 1 per cent solution of barium chloride is placed in two weighing bottles (*A* and *B*) of 25 c.c. capacity. One c.c. of the blood serum is then delivered under each of the solutions in the weighing bottles, and to *A* is then added 3.5 c.c. of 10 per cent ammonia solution. Both bottles are firmly stoppered. The bottle *A* is placed on a mechanical shaking apparatus. The bottle *B* is placed on a water bath, and the temperature kept at 60° C. for about fifteen minutes. By thus warming, any carbamate which the solution may contain is converted into carbonate.¹ The bottle *B* is then cooled, and 3.5 c.c. 10 per cent ammonia added; it is then placed on the shaker. After shaking for half an hour, the contents of the weighing bottles are transferred to centrifuge tubes which are tightly corked, and placed on a rapid centrifuge for about fifteen to twenty minutes. Seven c.c. of the supernatant fluid (corresponding to 0.5 c.c. serum) from each tube is then transferred to the gas bottle of the Barcroft-Haldane apparatus. An amount of a saturated watery solution of tartaric acid, almost sufficient to produce neutralization — determined by previous titration — is then delivered underneath the fluid in the bottles, and 0.25 c.c. of tartaric acid solution is placed on the spoons. The bottles are then attached to the manometers, — which, for convenience, are marked *A* and *B*, corresponding to the bottles, — and the temperature of the water bath registered on a thermometer reading one-tenth of a degree, brought to a fixed point near that of the temperature of the air of the room.² The temperature in the bottles having become constant, — as determined by the manometers, — the acid in the spoon is spilled into the

¹ Where the presence of a considerable amount of carbamate is expected — as after injecting carbamates into the circulation, or where carbamates have been directly added to the blood, etc. — the serum used in the control *B* should be some of the normal serum of the same animal, removed before the injection or addition of carbamates is made.

² To stir the water in the water bath we have used a mechanical stirrer connected with a water motor.

fluid in the bottles, and these are vigorously shaken. When all the gas has been expelled, the bottles are replaced in the water bath and cooled to the previous temperature. The meniscus of fluid in the manometers is then adjusted and read, and the calculation made as follows: The reading in the manometer *B* is deducted from that of *A*,¹ and the product multiplied by the capacity of the gas bottle and tubing. This latter value, divided by 10,000,² gives the number of cubic centimetres of carbon dioxide gas derived from carbamates.

To calculate how much calcium carbonate this represents, the above result should be multiplied by 3.8. 1 c.c. carbon dioxide equals 0.0038 gr. calcium carbamate (approx.).

A small positive reading is frequently obtained from the control *B*. This may be due to the presence of carbonates formed from the air during transference of the fluid to the gas bottle, or to the formation of carbamates by the action of the ammonia on the carbonates, or to the incompletely precipitated carbonates of the serum.

The most probable of these causes is the formation of carbonates from contact with air. A mixture of the reagents alone, treated exactly as above, but with less ammonia, gave on three different occasions readings of 7 mm., 6 mm., and 6 mm., and a saturated baryta solution alone, 8 mm. and 6 mm. With the same percentage of ammonia as above, the readings were -19 mm. and -18.5 mm.

Whether this be the correct explanation or not is of little importance for our purpose, for in any case the same reaction will take place in both bottles. That none of this gas is derived from unprecipitated carbonates of the blood serum is proved by the fact that the reagents alone give a similar reading. That the difference between the readings of *A* and *B* really corresponds to carbamate, can be further shown by examining, by the above method, blood serum which has stood for some time, and in which, therefore, all carbamate will have become converted into carbonate. In such a case these readings were found to be in one experiment: -19 mm. for *A*, and

¹ The bottles *A* and *B* are practically of the same capacity, so that this deduction is allowable.

² The fluid in the manometers is a solution of chromic acid of 10.30 specific gravity. If water were used in the manometers, the factor for division would be the barometric pressure in millimetres of water, viz., 10,300; but by having the fluid in the manometer of the above specific gravity, adjustment of the decimal is all that is required. The use of the apparatus in this way was suggested to us by Dr. Barcroft.

—15.5 mm. for *B*. It will be noted that in these cases a marked negative reading was obtained.

To demonstrate the value of this method we offer the following results: To 1 c.c. of blood serum diluted with ammonia water to make 7 c.c. of fluid containing 5 per cent ammonia was added 1 mg. calcium carbamate.¹ When the carbamate had all dissolved, 7 c.c. of

TABLE IV.

I. No.	II. Approximate amount of carbamate added. ¹	III. Carbon dioxide obtained from half of II (directly estimated).	IV. Pressure of gas in supernatant fluid of <i>A</i> .	V. Pressure of gas in supernatant fluid of <i>B</i> .	VI. Gas of carbamate (difference between IV and V).		VII. Difference between amount found and amount added.
					Pressure.	cubic centimetres.	
1	gram 0.0011	c.c. 0.1207	mm. 55.5	mm. 17	mm. 38.5	0.131	c.c. 0.0103
2	0.0012	0.1310	omitted in notes		39.5	0.134	0.0030
3	0.0012	0.1310 ²	55.5	18	37.5	0.129	0.0020
4 ³	0.0011	0.1207 ²	40.0	7	33.0	0.122	0.0013
5	small amt.	0.1510	49.5	6	43.5	0.148	0.0030
6	"	0.1235	24.0	—8.5	32.5	0.112	0.0110
¹ Calcium carbamate was used throughout. ² Those values were taken on the basis of estimation in Nos. 1 and 2. ³ In Nos. 1, 2, and 3 no barium chloride was used.							

a clear saturated solution of baryta was added, and the weighing bottle corked and placed on the shaking apparatus. The rest of the process was carried out as described above. Into another weighing bottle were placed the above reagents and serum, but no carbamates.

¹ The weighing of such a small amount is naturally subject to considerable inaccuracy.

THE INFLUENCE OF FEVER ON THE REDUCING ACTION OF THE ANIMAL ORGANISM.

By C. A. HERTER.

IN a previous paper¹ I described the effects of a depression of the body-temperature on the reduction of methylene blue to leucomethylene blue, and laid special emphasis on the impaired reducing action which is demonstrable in the muscles and gray substance of the central nervous system. Since making these observations I have carried out other experiments of a similar character upon animals in which the temperature had undergone an elevation. The outcome of these experiments appears of sufficient interest to justify me in placing on record some of the details.

The main result of the experiments which form the subject of the present paper is the demonstration of a greatly increased reducing action during fever by means of suitable color indicators (methylene blue or indophenol). The result is, as was anticipated, the opposite of that obtained by the experiments concerned with subnormal temperatures. It was found, however, that the acceleration of the reducing action which is occasioned by a rise of 3° or 4° C. gives indications of being as pronounced as the diminution in reducing action that is demonstrable by a depression of temperature through 8° or 10° C. Hence the study of cell-reductions in fever is a peculiarly satisfactory field for the application of intravital colorimetry.

The contrast between the fever animals and the controls can easily be made striking. The best results have been obtained under conditions slightly different from those recommended for the study of the effects of cold.

In most of the experiments upon the action of fever, the rabbits have been infused intravenously with a 25 per cent solution² of methy-

¹ HERTER: This journal, 1904, xii, p. 128.

² At first, the percentage of salt in the solution was 0.85 per cent, but as this concentration salts out some of the dye at low room-temperatures the sodium chloride was reduced to 0.4 per cent.

lene blue at the rate of 1 c.c. per minute, and from 50 to 75 c.c. have usually been introduced. Instead of waiting five or ten minutes after the close of the infusion before examining the organs, the animals have been killed (by ventricular incision), without delay, at the end of the infusion. These changes have been found advisable owing to the rapidity of reduction at fever heat.

Two different methods of elevating the temperature have been employed. In one set of cases the animal was enveloped in cotton batting, and an incandescent electric light was passed over the surface until the temperature of the body had been gradually elevated to the desired point, where it was without difficulty maintained during the infusion. In another set of experiments, the temperature was raised by means of hog-cholera infection. The infection was induced by means of cultures of hog-cholera of known virulence furnished me by Prof. Theobald Smith. One of the cultures sent me by Professor Smith gives rise to a temperature of 42° or 43° C. on the fourth or fifth day after inoculation with $\frac{1}{2000}$ c.c. of a twenty-four hour bouillon culture. Higher temperatures are observed later in the course of this fatal infection, but there appears to be no advantage in employing them in connection with the present investigation.

In order to illustrate the effect of elevation of temperature upon reduction in the organism, I shall give two typical protocols, one from an experiment in which the rise was caused by the external application of heat, and another in which the fever was of infectious origin.

An examination of the results recorded in Experiment 1 plainly shows the accelerating effect of elevation of temperature on the reduction of methylene blue by various types of cells. The difference in color between the corresponding parts in the two animals is the expression of the difference in reducing activity. The first evidences of this difference appeared during the infusion of the dye in the readily visible pectoral muscles, which were bared for purposes of observation. After the close of the infusion the animals were killed promptly enough to enable one to obtain an idea of the state of reduction at the end of life. The differences in color were especially striking in the brain, skeletal muscles, heart, spleen, pancreas, and liver. In all these situations reduction was more intense in the superheated animal than in the control, and, excepting the kidney, it may be stated here that this was the case not only in this experiment, but also in all the experiments of the sort that were conducted. It should be noted also that the warmed rabbit secreted less blue by

the urine and by the stomach than did his fellow, and further, that the blood in the warmed animal contained less blue than the control. These are features of interest in relation to the actual distribution of

EXPERIMENT I.

NORMAL RABBIT (CONTROL).

Temp. 38°-39° C. Weight, 1565 gms.
Received intravenously 42 c.c. of 0.25 per cent methylene blue solution in 42 minutes. Killed about 3 minutes after infusion's close.
Muscles: pectorals bluish during life, quick reduction after death, the muscles of back of neck, however, remaining blued for some time.
Application of H_2O_2 shows considerable leuco-blue in muscles.
Heart found unblued (ventricles).
Brain: blue (turquoise).
Spleen: blue.
Pancreas: moderately blue.
Suprarenal: colorless, does not blue on addition of H_2O_2 .
Kidneys: blue.
Urine: scanty, deep blue.
Liver: purple, contains unreduced blue.
Liver pulp contains considerable paired leuco-methylene blue.
Bile: moderate amount; contains considerable methylene blue; paired leuco-methylene blue, moderate amount.
Blood: considerable methylene blue; considerable leuco-methylene blue; paired leuco-methylene blue, scanty.
Stomach: much blue on surface of contents.

SUPERHEATED RABBIT.

Temp. 42°-43° C. Weight, 1510 gms.
Received intravenously 42 c.c. of 0.25 per cent methylene blue solution in 42 minutes. Killed about 3 minutes after infusion's close.
Muscles: pectorals quite colorless during life. Muscles of back of neck completely reduced.
Application of H_2O_2 shows considerable leuco-blue in muscles, more than in control muscles.
Heart found unblued (ventricles); contains considerable leuco-blue, more than control.
Brain: unblued. Oxidation with H_2O_2 shows that the brain contains more leuco-blue than the control.
Spleen: unblued; on oxidation with H_2O_2 blues to about the color of control.
Pancreas: pale blue.
Suprarenal: colorless; blues, cortex and medulla, on addition of H_2O_2 .
Kidneys: uncolored.
Urine: scanty, moderately blue (greenish)
Liver: red; complete reduction of blue.
Liver pulp contains considerable paired leuco-methylene blue.
Bile: scanty; contains little methylene blue. Moderate amount of paired leuco-methylene blue (slightly more than control).
Blood: little or no methylene blue; considerable leuco-methylene blue; paired leuco-methylene blue, scanty.
Stomach: little or no blue on surface of contents.

dye in the body. The criticism might be made that the differences in color in the two animals were possibly referable to variations in the distribution of the dye rather than to unequally energetic reduction. In answer to such a criticism it might be said that, if one

organ or a group of organs in the warmed animal received less blue than the corresponding organ or group of organs in the control, there would necessarily (since the infusions are of the same magnitude) be a compensating difference in the opposite direction, in some other part of the body, whereas in reality the color contrasts between the organs of the two animals were so widespread that they may be designated universal. Further and more convincing evidence is, however, not wanting to show that the color differences are not dependent on unevenness of distribution. Experiments made with eosene (which undergoes no reduction or other demonstrable chemical change in the body) showed that the distribution of this dye is essentially the same in the organs of the normal rabbits as in the corresponding organs of animals in which the temperature has been raised, — the conditions of infusion having, of course, been kept the same in these experiments. Finally, the ease with which methylene blue undergoes oxidation gives us a direct method of determining the actual distribution of the dye in the organism, at least in the case of the central nervous system and the muscles. If we have any doubt whether the brain has taken up as much dye as its fellow, we have only to pour upon a freshly cut surface a solution of hydrogen peroxide, when the leuco-blue or reduced blue is rapidly oxidized to the dye itself. By means of this method, it was easy to show that the brain and muscles of the warmed animal held not less but *more* dye than the corresponding parts of the normal rabbit.

When we come to the other organs, especially the liver and kidneys, the conditions are not so simple, for they are complicated by the occurrence of a synthesis or pairing of the methylene blue with some unknown constituent of the cells. The substance thus formed I have called paired leuco-methylene blue, or more briefly "paired substance." The important characteristic of this substance in the present connection is that it fails to be oxidized (in neutral or alkaline medium) to methylene blue, and hence escapes recognition in the simple process of oxidation. The dye can, however, be unmasked by boiling the organ pulp with a dilute acid — acetic being perhaps the best for this purpose. Until I recognized the pairing process in the liver and elsewhere, it was impossible to account for the disappearance of the infused methylene blue, since it was evident that the sum of the simple leuco-blue and the unreduced blue was far from being equal to the amount of dye infused.

It would be incorrect to give the impression that the total amount

of methylene blue in the febrile organs was always or even usually in excess of the amount in the corresponding organs of the controls. Even in animals of the same temperature, infused under the same conditions, the brain and muscles (structures holding little or no

EXPERIMENT 2.

NORMAL RABBIT (CONTROL).

Temp. 39° C. Weight, 1450 gms.

Infused intravenously 31 c.c. 0.25 per cent methylene blue solution, 1 c.c. per minute. Killed at close of infusion.

Muscles: blue during life. At death, blue was rapidly reduced. With H_2O_2 moderately blue.

Heart: Ventricles blued.

Brain: almost completely reduced; blues deeply on exposure to air; with H_2O_2 very deep blue.

Spleen: blue.

Pancreas: not noted.

Suprarenals: not noted.

Kidneys: unblued; considerable leuco-blue in cortex.

Urine: none.

Liver: bluish; leuco-blue, considerable; pulp yields considerable paired leuco-methylene blue.

Bile: quantity moderate; some methylene blue; some paired methylene blue.

Blood: serum blue; very little leuco-blue; considerable paired leuco-methylene blue.

Stomach: contains considerable blue.

INFECTED RABBIT (HOG CHOLERA).

Temp. 40.5° C. (4 days after inoculation).¹

Weight, 1435 gms.

Infused intravenously 31 c.c. 0.25 per cent methylene blue solution, 1 c.c. per minute. Death at close of infusion.

Muscles: no bluing during life; with H_2O_2 less blue than control.

Heart: ventricles unblued.

Brain: completely reduced, but blues only slightly on exposure to air; with H_2O_2 blues somewhat less deeply than control.

Spleen: red.

Kidneys: blue; papillae and medulla blue on exposure to air.

Urine: none.

Liver: gray; contains little or no leuco-blue; nearly all blue exists as paired leuco-methylene blue; quantity about same as in control.

Bile: scanty; no methylene blue (as such); very little paired leuco-methylene blue (*i. e.* no secretion).

Blood: serum uncolored; little or no leuco-blue. All dye is present as paired leuco-methylene blue.

Stomach: contains very little blue.

¹ The temperature of this animal had been higher, but it fell when the animal was placed on the holder, as is often the case.

paired substance) often show, on oxidation with hydrogen peroxide, moderate inequalities in the content of dye. In the case of experiments made at an elevated temperature, the greater amount of dye is found sometimes in the muscles and brain of the control, sometimes in the structures of the superheated mate. The evidence of increased reduction is, of course, especially pronounced in those cases where the

brain and muscles not merely are colorless, but contain more total dye-stuff than their fellow-organs.

In essential respects the conditions in Experiment 2 after infusion resembled those found in Experiment 1; that is to say, the various parts, including brain, muscles, heart, spleen, and liver, showed the presence of less blue in the febrile rabbit than in his fellow. The differences were, however, less pronounced than in Experiment 1, and this is hardly surprising, inasmuch as the disparity in temperature was only $2.5^{\circ}\text{C}.$; whereas, in Experiment 1 the inequality amounted to probably not less than $4^{\circ}\text{C}.$ In other observations in animals infected with hog cholera, higher temperatures were obtained, and in such instances the results were indistinguishable from those seen in animals whose temperature had been raised to an equally high level by external application of heat.

There is usually a distinct difference between the color of the blood of the febrile animal and that of the control, the former containing less methylene blue as such and more leuco-methylene blue. The bile in the gall-bladder of the febrile animal is almost always scanty, and contains less methylene blue than the bile from the control. The stomach also shows a diminished secretory activity when the temperature is elevated, for little or no blue finds its way into the interior of this organ, whereas in the control animals the food is found covered by a layer of mucus mixed with unreduced blue. Once, however, I found these conditions reversed. As regards the quantity and character of the urine secreted during the infusion the results are extremely variable. I have generally found that the febrile urine contains rather less methylene blue and more leuco-methylene blue than the urine from the normal control, but there may be more blue in the febrile urine. It seems probable that the wide variability noted in wholly normal animals with the same temperature affords a sufficient explanation of these irregularities. Occasionally, as in Experiment 2, there is no secretion during the infusion. In general, it can be stated that the quantity of dye recovered in the urine is too small to exert a material influence on the quantity or distribution of that which remains in the organism.

The observation that methylene blue is capable of serving as an admirable indicator of the acceleration of reduction resulting from an elevation in temperature naturally suggested that the various kinds of cells in which the reducing powers had been watched during life might be advantageously studied *in vitro* in respect to this activ-

ity. The prospect of being able to study individually the properties of different tissues, unhampered by the uncontrollable and unmeasurable interactions characteristic of the living organism in its entirety, made it appear worth while to seek a method of conducting such experiments under conditions permitting a measurement of the processes in question.

After some unsuccessful trials, the following method of measuring approximately the reaction velocity of reduction was adopted. The tissues to be studied (liver, kidney, muscles, etc.) were taken from a dog or rabbit (which had been bled) and subdivided in an ordinary meat machine. A finer degree of subdivision was secured by trituration with fine sand. Definite weights of tissue thus prepared and mixed with sand were placed in thin walled capacious test-tubes of hard glass, one and one-quarter inches in diameter, to which a fixed volume of distilled or tap water was added. For example, to 2 or 4 gms. of triturated liver was added usually 25 gms. of water. To this mixture was added at the proper time 1 c.c. of a weak methylene blue solution (0.025 per cent in distilled water) at the proper temperature. In order to secure better contact of the dye with the particles of tissue, a constant stream of washed and neutralized nitrous oxide gas was passed through the mixture.¹ As our gas liberated a very slight quantity of oxygen, its action, aside from a mechanical one, must have been to slightly retard reduction. Carbon dioxide was abandoned because of the disturbing effect exerted by its acid properties.

The temperature within the tubes was easily regulated, and there was no difficulty in keeping their contents in a practically anaërobic state. In order to insure the rapid and thorough mingling of the dye with the remaining contents of the tube, it has been found helpful to inject the methylene blue directly into the rubber delivery tube through which the gas enters the lowest part of the tube. The reduction tube is prepared for action by allowing the nitrous oxide to bubble actively through it for five minutes before the dye is introduced.

As regards the endpoint of the reaction, it must be said that this is not always as sharp as could be desired, and that it seldom happens

¹ A drawing of the apparatus will be given in a subsequent paper. Each tube is fitted with a rubber cork with three openings, one for the tube delivering the gas, one for a thermometer, and a third for the exit of the gas. Two tubes are generally operated simultaneously. The desired temperature within the tubes is secured by their immersion in a beaker of water.

that the original color of the tube is regained, even through prolonged action of the tissues on the blue. For this reason, it has been found best to take the reading when the last trace of the green-blue disappears from the mixture in the tube. Where control observations are being made, it is usually not difficult to fix upon an arbitrary endpoint which is the same for both tubes.

By means of the method thus indicated, it has been possible to study the influence of many conditions and substances upon the velocity of reduction, including the action of acids and alkalies, the ions of neutral salts, the effects of colloidal solutions,¹ etc. At present reference will be made only to a few typical observations on the influence of temperature.

The following are a few examples of the influence of temperature and mass of tissue (rabbit's liver):

- 1 gm. liver + 1 c.c. blue solution + 25 c.c. H₂O at 38° C. reduced in 24½ minutes.
- 1 gm. liver + 1 c.c. blue solution + 25 c.c. H₂O at 43° C. reduced in 18 minutes.
- 2 gms. liver + 1 c.c. blue solution + 25 c.c. H₂O at 38° C. reduced in 3½ minutes.
- 2 gms. liver + 1 c.c. blue solution + 25 c.c. H₂O at 43° C. reduced in 2 minutes.

Material from the liver of another rabbit gave the following results:

- 1 gm. liver + 1 c.c. blue solution + 25 c.c. H₂O at 38° C. reduced in 8 minutes.
- 1 gm. liver + 1 c.c. blue solution + 24 c.c. H₂O + 1 c.c. 0.85 per cent NaCl solution at 38° C. reduced in 8 minutes.
- 1 gm. liver + 1 c.c. blue solution + 25 c.c. H₂O at 43° C. reduced in 4 minutes.
- 1 gm. liver + 1 c.c. blue solution + 24 c.c. H₂O + 1 c.c. 0.85 per cent NaCl solution at 43° C. reduced in 5 minutes.
- 2 gms. liver + 1 c.c. blue solution + 25 c.c. H₂O at 38° C. reduced in 1¼ minutes.
- 2 gms. liver + 1 c.c. blue solution + 25 c.c. H₂O at 43° C. reduced in 45 seconds.

With larger quantities of triturated tissue, say 4 or 5 grams of liver, reduction may occur so rapidly that it is difficult to measure the difference in velocity at 38° and 43° C.

At one time it seemed to me that our experiments indicated a greater reaction velocity than is characteristic of simple chemical

¹ An interesting field for observation is a comparison of the reducing action of different structures in the same organism, and of the corresponding structures in different groups of animals. In the present experiments the great activity of liver and kidney as compared with brain and muscle is an obtrusive feature. The superiority of the dog's liver over the liver of the rabbit in regard to the power of reduction can be easily shown. The action of poisons has not yet been taken up with this method, except to a limited extent.

reactions; but at present it is doubtful if that view can be maintained. In order to gain information as to the influence of temperature on the velocity of reduction in the absence of organized material, the tubes were filled with a solution of 10 c.c. of (approximately) 3.71 per cent glucose solution, 1 c.c. methylene blue solution, 2 c.c. $\frac{1}{10}$ sodium hydrate solution, and 12 c.c. water. At 38° C., reduction occurred in nine and one-quarter minutes; at 43° C. in six minutes; at 48° C. in four minutes. The results thus derived are apparently of the same order as those obtained with living tissues.

It is clear that many factors influence the velocity of reduction by living animal tissues. In some of the above experiments the effect of a small amount of sodium chloride is seen,¹ and it can be shown that there is a high degree of sensitiveness to the presence of hydrogen and hydroxyl ions. The differing activity of the livers of different individuals *in vitro* is a striking thing, and reminds one of the individual differences noted in the course of intravital infusions. A factor which may become disturbing in test-tube experiments is the postmortal decline in activity of the cells. In the case of muscle, especially rabbit muscle, this is rapid. The liver, however, usually retains a high grade of reducing activity for several hours after death.

It is believed by the writer that the demonstration of the action of fever on the organism by the intravital method described in these pages will not only prove serviceable in the study of the pathology of fever, but will afford a highly instructive object lesson in class work. Probably the method of studying reduction *in vitro* will prove of considerable use in the analysis of the many factors that affect the process of reduction. I hope before long to report on the behavior of different kinds of living tissue under physiological and pathological conditions.

I wish to acknowledge the valuable assistance I have received from my laboratory assistant, Mr. Edward O'Brien, in the conduct of these experiments.

¹ At 38° C. sodium chloride, in larger amount than in the experiment given above, was found to accelerate reduction, but at higher temperatures it was frequently observed to exert the opposite influence.

THE PRODUCTION OF FAT FROM PROTEID BY THE BACILLUS PYOCYANEUS.

BY S. P. BEEBE AND B. H. BUXTON.

[From the Department of Experimental Medicine in the Cornell University Medical College, New York City.]

THE production of fat from proteid has been among the most interesting of physiological and pathological problems. In this paper are given the conditions under which such a production due to the growth of a microorganism was found to take place. That certain microorganisms form fat is admitted, notably the bacillus tuberculosis, the intracellular fat of which has been the subject of recent studies by Bulloch and Macleod,¹ and Levene.² In the present instance it is the belief of the writers that the conditions under which the fat is formed are somewhat more definite than in the previous instances.

A stock culture of the bacillus pyocyaneus, of unknown origin, was observed to form a thick wrinkled pellicle on the surface of the agar or broth in which it was growing. On making a hanging drop preparation from the pellicle, it was seen to consist of masses of bacilli among which bundles of long needle-shaped crystals were frequent.

The only paper in the literature which could be found referring to the formation of crystals by the bacillus pyocyaneus was a note by Dorset in the *Centralblatt für Bakteriologie* (Vol. 20, 1896, p. 217). Agar cultures of Dorset's bacillus, which was isolated from a guinea-pig killed by inoculation of oleomargarine, formed small needle-shaped crystals which penetrated beneath the surface of the agar, — tests proving conclusively that these crystals were not due to mere drying-out of the medium.

Chemically, the crystals were shown to be phosphates, principally of calcium with traces of magnesium, and were apparently "due to a separation of the phosphates normally present in the medium, caused

¹ BULLOCH and MACLEOD: *Journal of hygiene*, 1904, iv, pp. 1-10.

² LEVENE: *Journal of medical research*, 1904, xii, pp. 251-258.

by the specific action of certain products of the bacillus." The disposition of the crystals in this case was very different from that in our cultures. Instead of crystals being formed in the agar, we find them only in the pellicle, in intimate connection with the bacilli. However, in view of Dorset's statements, we subjected our crystals to a preliminary test for phosphates with negative results.

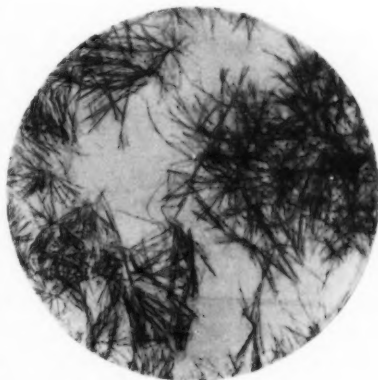
Further examination showed that:

1. The crystals are soluble in alcohol, ether, chloroform, and to some extent in petroleum ether.

2. They give staining reactions for fats with osmic acid and sudan III.

3. The alcoholic solution on saponification affords a soap from a solution of which fatty acids can be precipitated by a mineral acid, and then extracted by shaking out with ether.

The photograph shows the similarity of the crystals with those of certain of the higher fatty acids, and the conclusion was soon reached that they must be of a fatty nature.



The next step was to prepare the fat in sufficiently large quantities for chemical examination; and, after trial of several methods,

the following was adopted as being the simplest and most effective.

Two litres of meat-extract broth with 3 per cent peptone are prepared at a time and distributed into eight 1000 c.c. Erlenmeyer flasks, 250 c.c. in each, a large surface for the formation of the pellicle being thus secured. After sterilization, the flasks are inoculated from an agar culture and allowed to stand, without being disturbed for two or three weeks, in the incubator. At the end of that time a thick wrinkled pellicle has formed on the surface, and with a little care the underlying fluid can be decanted off, leaving the pellicle lying intact at the bottom of the flask.

After drying for two or three days, each flask is filled with chloroform until the pellicle is entirely covered, and allowed to stand for twenty-four hours with occasional shaking. Usually two flasks at a time were so treated, the chloroform being then poured into the next

two. After all the flasks have been thus treated, the chloroform, which has assumed a yellowish tinge, is filtered to free it from the fragments of the pellicle remaining, consisting of masses of bacteria.

The chloroform is then mostly recovered by distillation, and the dark-brown concentrated residuum evaporated to dryness at 100° C. in a current of hydrogen gas, the flask cooled over sulphuric acid and weighed. The dried residue, of a yellow-brown color, is dissolved in ether, and the solution kept in a stoppered bottle for future use.

In this way we collected about 10 grams of fatty substances from 30 litres of broth culture, — an amount considered sufficient for preliminary examination. The quantity of fat obtained from each litre was about 0.3 to 0.4 gram after we had once hit upon the best method of procedure.

Chemical examination. — The chemical examination of the fat has been rendered difficult because of the small quantity at our disposal.

The following fat constants were determined: (1) Melting point, 70°. (2) Acid number, 47. (3) Saponification number, 94. (4) Iodine number, 70.

(1) **Melting point.** — It must be noted that the fat does not have a sharp melting point, nor does it, even at a much higher temperature than 70°, become fluid as the ordinary fats do, but softens to a thick, syrupy condition.

(2) **Acid number.** — This number gives the milligrams of potassium hydroxide required to neutralize 1 gram of the fat. Forty-seven, as the acid number, indicates that a considerable portion of the substance is free fatty acid. The fat from the tubercle bacillus has an acid value of 23. The ordinary fats vary from 0.3 to 9 in acid value.

(3) **Saponification value.** — This gives the number of milligrams of potassium hydroxide required to saponify 1 gram of the fat. The value in this instance is low, indicating the presence of a considerable amount of unsaponifiable substance. The tubercle bacillus fat had a saponification value of 60.7, and was also found to contain a large amount of unsaponifiable substance. The saponification value of a few common fats may serve for comparison: cocoa butter, 192; mutton tallow, 195; horse fat, 195; goose fat, 195.

(4) **Iodine number.** — The iodine number gives the percentage of iodine which a fat may take up.

The unsaturated fats under certain conditions readily take up definite amounts of iodine so that this number serves as a convenient means of identifying them. The number, 70, is somewhat higher

than that of most solid fats. In animal fats, the iodine is absorbed by the oleate radical, but there are other unsaturated fatty acids which absorb even more than oleic acid.

A considerable portion of the fat was saponified by one of the common methods, with alcoholic potash, and the resulting solution of soap was evaporated to dryness after adding 5 grams of sodium chloride. The dried mass was powdered and extracted with ether. After evaporation of the ether a waxy substance was left which was found to weigh 78 per cent of the fat used for saponification. In order to determine if a portion of this wax could be saponified by more drastic methods, it was dissolved in absolute alcohol, and the solution slowly saturated with metallic sodium. The alcoholic solution was boiled for four hours, evaporated to dryness, the residue taken up in water and shaken out with ether. None of the wax had been saponified. The wax does not dissolve readily in ether, and scarcely at all in petroleum ether; alcohol and chloroform are its best solvents. It gives none of the color reactions for cholesterin. It stains with osmic acid and absorbs iodine; its iodine number was found to be 60.6. When the wax is boiled with acetic anhydride, it dissolves readily, but crystallizes out to some extent on cooling. Its behavior in this respect is like that of an alcohol.

The soap from the saponification of the original fat yielded a fatty acid having a low melting point, 41° . (The fatty acid was probably not pure enough to have much reliance placed on this figure.) It absorbed iodine and stained with osmic acid.

The question now arises as to the origin of the fats. Sugar-free meat broth and sugar-free extract broth with varying quantities (1 to 10 per cent) of Witte's peptone were prepared, and in these the crystals were readily formed.

Free sugar, therefore, could not be the source of the fat. On sugar-free solutions of nutrose and casein there was no development of the pellicle, so it might be supposed that the fat is derived from the carbohydrate group contained in the albumoses of the peptone (so-called), but the amount formed is so great (about 0.35 gram in 1000 c.c. of 3 per cent peptone broth, or in other words over 10 per cent of the peptone used) that the carbohydrate nucleus of the synalbumose could hardly account for all of it.

That the crystals could not be formed from fat pre-existing in the broth was made evident by extracting 1000 c.c. of the latter with ether. The merest trace of ether-soluble substances could be found.

Again 50 grams of peptone extracted with chloroform afforded negative results.

During the growth of the bacillus the medium becomes very alkaline, the alkalinity being due almost entirely to the presence of free ammonia. Of the total nitrogen found in one case, 33.7 per cent was split off by boiling with magnesium oxide. Obviously during the growth of the bacillus there is much ammonia split off from the proteids, and the resulting products may become oxidized to fats. It seems probable, therefore, that the fat is formed, at any rate partly, by oxidation of fragments of the albumoses and peptones apart from any carbohydrate nucleus they may contain.

That it is an oxidation process appears more than likely from the fact that the crystals are formed solely in the surface pellicle. Again under anaërobic conditions the bacilli grow sparingly, and no trace of pellicle or fat crystals is formed.

In addition to the fats a considerable amount of a mucinous substance is formed during growth. Although this substance gives certain reactions for mucin, such as precipitation by acetic acid and solubility in alkaline solution, yet, strange to say, we have been unable to detect the presence in it of a reducing substance except in mere traces, and that only on complete destruction of the albumin molecule after prolonged boiling in weak acid (2.5 per cent H_2SO_4).

Of those who have studied the mucin of the bacillus pyocyaneus, Rettger¹ found it yielded a compound which reduces Fehling's solution, while Charrin and Desgrez² obtained a reducing substance in two cases, a third yielding negative results. Since the mucinous substance, therefore, does not appear to call for any carbohydrate, we may relegate all of the carbohydrate nuclei of the albumoses to the formation of the fat; but even so, as already remarked, there could not be sufficient to account for all of it.

We expect to study more closely the ammoniacal, mucinous, and wax-like substances of our cultures and make them the subject of a future paper.

¹ RETTGER: *Journal of medical research*, 1903, p. 102.

² CHARRIN and DESGREZ: *Comptes rendus de l'académie des sciences*, 1898, cxxviii, p. 596.

FURTHER EVIDENCE OF THE NERVOUS ORIGIN OF THE HEART-BEAT IN LIMULUS.

By A. J. CARLSON.

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IN a previous paper¹ I have shown that the heart of *Limulus* contradicts the myogenic theory, both as regards the origin of the beat and as regards the tissues concerned in co-ordination or conduction in the heart. The experiments reported in that paper were performed at the Marine Biological Laboratory, Woods Hole, during the early part of June, but the work was continued till September, with the result of not only completely verifying the earlier observations, but also of obtaining data which place related questions of heart-physiology in a new light. This refers especially to the nature of cardiac inhibition and the nature of the action of drugs on the heart. As a preliminary to taking up those questions, the following observations may be recorded as further evidence of the nervous origin of the heart-beat:

I. DEGREE OF AUTOMATISM OF THE DIFFERENT REGIONS OF THE HEART.

It was stated in the previous paper that any segment of the heart will continue to beat rhythmically for some time after being isolated from the adjoining segments by transverse lesions, provided the nerve-cord is left intact in the segment. It was noted, however, that the three anterior segments may not beat spontaneously after such lesions. In the experiments during July and August spontaneous contraction in either of the first three segments, when isolated, were observed only in four out of sixty hearts tested. This refers to hearts from adult specimens. The hearts from young specimens worked on shortly after being brought into the laboratory usually exhibited a greater degree of automatism of the anterior or aortic end, just as did

¹ CARLSON: This journal, 1904, xii, p. 67.

the hearts of adults worked on in the early part of June. This slight difference between the specimens worked on in June and those used later in the season was probably due to a poorer state of nutrition and general condition of the animals later in the summer. The adult specimens are obtained in abundance only during the breeding season in May, when they are collected and kept during the summer in aquaria anchored in the bay. The animals keep well in this confinement; but as they are not fed, or do not take food, it is evident that the condition of nutrition grows steadily poorer as the season advances.

But even in young specimens, or in adult specimens in good condition, the greatest automatism is exhibited by the posterior two-thirds, or more correctly by the middle third of the heart. The anterior end of the heart is the aortic end, the posterior third or two-thirds of the heart correspond to the venous end. We can thus perform the Stannius experiment on the *Limulus* heart. The heart is prepared by carefully freeing the lateral nerves and the nerve-cord from the heart in the third or fourth segments, so that they may be severed transversely without injury to the heart-muscle. The lesion of the nerves and the nerve-cord in this region does not affect the rhythm of the posterior or venous end of the heart. Anterior to the lesion the rhythm ceases temporarily in every case, and it may or may not be resumed, usually the latter. Conduction takes place in the nervous elements, hence any rhythm anterior to the lesion of the nerves and the nerve-cord is not due to transmission of the contraction from the posterior end of the heart, but must be generated in the heart anterior to the lesion.

Thus we find in the heart of *Limulus* a condition similar to that in the vertebrate heart, the venous end of the heart exhibiting the greatest automatism, the aortic end the least or no automatism. On the myogenic theory, this condition in the heart of *Limulus* would be explained as in the vertebrate heart by the subsidiary hypothesis that the muscle at the venous end retains more of its embryonic character, and is therefore more automatic; but when this hypothesis is tried in the case of the *Limulus* heart, it is found wanting, for after extirpation of the nerve-cord or ganglion on the dorsal side of the heart, the heart-muscle exhibits the same degree of automatism in all regions of the heart, that is, no automatism at all.

In the distribution of the ganglion cells, we find still another similarity between the heart of *Limulus* and that of vertebrates, namely,

that the regions of the heart exhibiting the greatest automatism have the greatest number of ganglion cells. In the *Limulus* heart, the ganglion cells are collected in the nerve-cord on the dorsal side of the heart. The nerve-cord is thickest in the middle third of the heart, that is, in the fifth, sixth, and seventh segments, which means a greater number of ganglion cells in this region. In the first three segments, the nerve-cord is very slender and made up for the most part of nerve-fibres. There are some individual variations in this regard. Thus in two specimens I found an unusual ganglionic enlargement on the nerve-cord at the level of the first pair of ostia. But the few ganglionic cells in the nerve-cord of the anterior segments can in most cases not maintain rhythmic activity when separated from the region of the cord having the greatest automatism, just as the tortoise ventricle, when severed from the auricles, does not beat except when stimulated artificially.¹ In all probability the ganglion cells (or at least some of them) situated in the aortic end of the heart are similar in function to the ganglion cells of the respiratory centres in the spinal cord of vertebrates in not being active under normal conditions except when being stimulated by the impulses from the centre of greater automatism.

There is still another similarity in the distribution of the ganglion cells in the heart of *Limulus* and in the vertebrate heart, namely, in their position with reference to the myocord. In the vertebrate heart, they are situated in the main on the surface of the myocard, and this is also their position in the *Limulus* heart.

The absence of automatism in the isolated anterior segments of the *Limulus* heart may thus be due, in part, to the absence of impulses from the cells of the cord having the greatest automatism, but it is also due to the severing of the nerve fibres which pass from the nerve-cord in the middle of the heart directly to the muscle of the anterior segments. These fibres reach the muscle along the lateral nerves, as well as in the nerve-cord itself, as can be shown by the following experiments. If the nerve-cord is cut transversely in the third segment, and the portion of the cord anterior to the lesion extirpated, this end of the heart continues to beat in perfect synchrony with the rest of the heart, but the strength of the contractions is reduced. This rhythm is due solely to the impulses from the cord posterior to the lesion reaching the muscle in the lateral nerves, for lesion of either nerve diminishes still further the strength of the beats, and lesion of

¹ MARTIN: This journal, 1904, xi, p. 103.

both nerves stops the rhythm completely. When both lateral nerves are cut transversely in the third segment, leaving the nerve-cord intact, the amplitude of the beats anterior to the lesion is similarly reduced; but in this case it is difficult to determine whether the rhythm now maintained is due to impulses from the nerve-cord behind the lesion passing to the muscle directly, or to the activity of the ganglion-cells situated in the nerve-cord of the anterior segments. Both factors are probably present. By a similar series of lesions, it can be shown that nerve-fibres from the ganglion in the fifth and sixth segments pass in the lateral nerves and the nerve-cord to the muscle of the posterior segments of the heart (seventh, eighth, and ninth segments). We must call in the aid of the histologists to determine the mechanism effecting the co-ordination between the impulses passing directly to the muscles in all parts of the heart from the middle region of the cord, and impulses reaching the same or adjoining muscle cells from the ganglion cells situated in the same segment. The co-ordination of the *Limulus* heart is to be compared to the co-ordination of the auricle or the ventricle, rather than with the co-ordination of the whole heart in the vertebrates. Assuming that the physiology of the ganglion cells in the vertebrate heart is the same as in the heart of *Limulus*, the mechanism of co-ordination of the latter is in all probability similar to that effecting co-ordinated activity of the ganglion cells scattered all over the myocord of the vertebrate auricle. There is some individual variation in the course of the fibres from the nerve-cord of the middle region to the muscle of either end of the heart, as determined by the effect of the lesions on the strength of the contraction. More fibres may pass in the left than in the right lateral nerve, or vice versa. It is rare that the majority of the fibres pass in the nerve-cord.

In all experiments involving the determinations of the rate and the strength of the beats, the ordinary graphic method was employed. For this purpose it is preferable to work on the excised heart. A recording lever may be adjusted to the heart *in situ*, but removing the heart from the body does not affect the rhythm, and in either case the heart is empty, the mere opening of the pericardial cavity preventing the entrance of blood into the heart. The contraction of the heart-muscle diminishes the diameter of the heart without changing its length, as the muscle cells are arranged circularly. For graphic registration, one needs only to secure one side, or rather lateral angle, in any region of the heart to a fixed support and connect

the opposite side with a recording lever. In case any of the first four segments are used for recording the contractions, the attachment of the heart to the support and the lever is most conveniently made by glass hooks in the lateral arteries in the manner shown in Fig. 10. When it is desired to make use of any of the middle or posterior segments, the hooks are fastened to the muscle directly. A record thus obtained represents the contractions of one, or part of one, segment only. The heart of large specimens is about 12 cms. long. The parts of the heart not connected with the recording apparatus were supported independently so that their contractions did not influence the lever. When the first or second segments were used for contraction, the posterior part of the heart was usually placed in a shallow dish filled with plasma, and thus supported on a level with the recording segment.

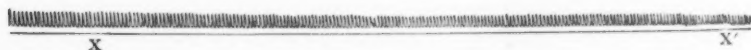


FIGURE 1.—Tracing of contractions of first segment. *X*, beginning of severing the connectives between the nerve-cord and the lateral nerves in the last segment, the lesion being continued forwards till at *X'* the nerve-cord is isolated up to the third segment. Diminution of contractions of the anterior segments following the lesion. Continued activity of the ganglion cells, although the nerve-cord is isolated from the greater part of the heart.

Tracings illustrative of the effect of the lesions of the nervous complex described above are reproduced in Figs. 1 to 3. The record in Fig. 1 is from the contraction of two anterior segments, the hooks being fastened in the first pair of lateral arteries in the manner shown in Fig. 10. At *X* the beginning of the lesion of the connectives between the nerve-cord and the lateral nerves was made in the seventh segment and proceeded anteriorly until at *X'* the nerve-cord was completely isolated from the lateral nerves up to the third segment. In this particular experiment that part of the cord was at the same time almost completely isolated from the heart-muscle, as the lesion of the nerves was effected by cutting through both the nerve plexus and the dorsal wall of the heart at either side of the cord and at a distance of 1 mm. from it. It is not possible to sever all the connectives in any other way. The nerve-cord must not be touched by the instruments, as that causes acceleration and inco-ordination of the rhythm. If the operation is made by a pair of sharp scissors, and the nerve-cord not touched, the whole ganglion may thus be isolated

from the heart-muscle, save in the first three segments, without greater change in the activity of the ganglion cells than that exhibited by the tracing in Fig. 1. It will be observed in this tracing that as the severing of the connectives between the nerve-cord and the lateral nerves proceeds forward, the strength of the contractions diminishes by degrees, until at *X'* the amplitude of the beats is only about half that of the original. This diminution in the strength of the beat does not imply any change in the activity of the ganglion cells. It is simply due to the severing of the nerve-fibres, and thus cutting off the impulses that pass in the lateral nerves from the ganglion in the middle region of the heart to the heart-muscle of the first two segments. The only change in the activity of the ganglion cells is the slight but steady increase in the rate of the beats. This is, without exception, the first result of the lesion, and in the majority of the preparations the acceleration was much greater than that shown in Fig. 1. In these preparations the acceleration was accompanied by inco-ordination, the beats soon became diminutive and the rhythm ceased. When the operation succeeds, as in Fig. 1, a strong and fairly regular rhythm of the first two segments is maintained for from ten to thirty minutes, but inco-ordination sets in before the final cessation of the rhythm. Now, this is the condition. The rhythm of the first two segments after severance of the lateral connectives up to the middle of the third segment is still caused by the activity of the ganglion cells situated behind this region of the heart, as is shown by the fact that the cross-section of the cord in the third segment stops this rhythm instantaneously. But behind the second segment the nerve-cord is completely isolated from the heart-muscle, save that portion of the dorsal wall immediately beneath the cord. Yet the activity of the ganglion cells goes on for some time, and, in some preparations, without change in rate or intensity. So far, then, the activity of the ganglion cells does not appear to be primarily dependent upon afferent impulses from the heart, although such afferent impulses are probably present under normal conditions, as will be shown later. It may be argued that the afferent impulses from that part of the heart-muscle still in connection with the ganglion are sufficient to maintain the activity of the ganglion cells, or that the severance of the nerves produces sufficient injury to cause a series of impulses in the nerve-fibres, which thus take the place of the afferent impulses present under normal conditions. The acceleration of the activity of the ganglion is probably due to stimulation from the

unavoidable handling, as well as to the chemical changes taking place at the cut end of the nerve-fibres.

The dependence of the rhythm of the anterior end of the heart on the nerve-cord in the middle region of the heart, and the function of the nerve-cord and the lateral nerves in affording paths for these nervous impulses, are clearly shown in the tracing reproduced in Fig. 2. This record is also from the contraction of the first two segments. The posterior part of the heart was supported in a dish filled with plasma. Prior to suspending the heart for graphic registration, the nerve-cord and the lateral nerves were isolated from the heart for a distance of 1 cm. in the third segment, so as to be readily severed with a pair of scissors without injury to the heart-muscle. At *a* the nerve-cord is cut transversely in the third segment. This is followed by acceleration of the rate and diminution of the strength of the beats. The acceleration is only temporary, the

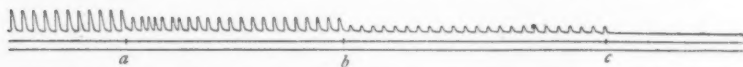


FIGURE 2.—Tracing of contractions of the first segment. *a*, cross-section of the nerve-cord in the third segment; *b*, cross-section of the right lateral nerve in the third segment; *c*, cross-section of the left lateral nerve in the third segment. Nerve-cord and lateral nerves isolated from the heart in the region of the lesions prior to the experiment, so that the lesions did not injure the heart-muscle.

diminution in the amplitude of the contractions is permanent. The acceleration is evidently due to the mechanical stimulation in cutting the nerve-cord, as the mere touching the cord produces the same results (Fig. 18). The diminution of the beats is due to elimination of a part of the impulses reaching the anterior end of the heart from the ganglion cells situated behind the lesion. At *b* the lateral nerve on the right side is cut in the third segment. This lesion does not affect the rate, but reduces the amplitude of the beats still further by eliminating all the motor impulses reaching the heart-muscle of the reacting segments through that nerve. At *c* the left lateral nerve is severed in the same region. In these lesions of the nerve-cord and the lateral nerves, the heart-muscle has not been touched, but all the nervous connections between the first two segments and the ganglion cells situated beyond the third segment have been severed, and in consequence of this the rhythm of the anterior segments ceases, in most cases permanently. The portion of the heart posterior to the lesion continues its rhythm unchanged, in the same

manner as do the sinus and the auricles of the frog after severance of the ventricle, except that the rhythm is temporarily accelerated on the cross-section of the nerve-cord, an acceleration clearly due to the mechanical stimulation.

The tracing in Fig. 2 shows a rather exceptionally equal distribution of the nerve-fibres in the nerve-cord and the lateral nerves. Frequently the section of one of the lateral nerves produced a much greater diminution of the beat than the section of the opposite nerve. In some specimens the greater part of the fibres appear to pass in the nerve-cord itself, as shown in Fig. 3. The individual variations

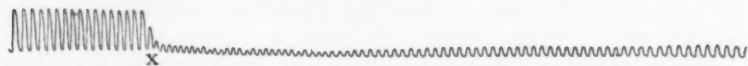


FIGURE 3.—Tracing from the first segment. *X*, nerve-cord cut in the third segment leaving the lateral nerves intact. Unusual diminution of the contractions on cross-section of the cord in that region.

can also be made out anatomically, the size and number of the connectives between the nerve-cord and the lateral nerves being either relatively few or greater on the one side than on the other.

II. THE PHYSIOLOGY OF THE CENTRIFUGAL NERVE-FIBRES FROM THE NERVE-CORD TO THE HEART-MUSCLE.

The preparation of the heart for the study of the function of the nerve-fibres passing from the cells in the nerve-cord to the heart-muscle is represented in Fig. 4. The lateral nerves are isolated up to the second segment, the median nerve-cord is extirpated, and the heart severed transversely in the middle of the second segment. By means of hooks in the first pair of lateral arteries, the isolated anterior end of the heart is connected with the lever for graphic registration. The electrodes may now be applied to the lateral nerves 5 to 6 cms. from the muscle. If it is desired to study the influence of the nerve-cord on the quiescent muscle, the nerve-cord may be left in connection with the first two segments, and severed in the fourth segment. After previous dissection of the lateral nerves, lesion of the nerve-cord in the fourth segment is usually followed by complete cessation of the rhythm of the first two segments. In the few preparations in which a feeble rhythm persists, it ceases after a few periods of stimulation of the nerve-cord with the interrupted current. When the nerve-cord is extirpated in the manner shown in Fig. 4, the

musculature of the first two segments remains perfectly quiescent. This preparation thus offers an unequalled condition for the study of the influence of the intrinsic heart-nerves on the quiescent heart-muscle.

It was stated in my previous paper that *the nerves passing from the nerve-cord to the heart-muscle are of the ordinary motor type*. This has now been further confirmed by aid of the graphic method. The heart-muscle responds to a single induced shock applied to either one or both of the lateral nerves, but the nerves possess a relatively low excitability to the induced current. The induced shocks must therefore be relatively strong to produce a contraction when applied to the

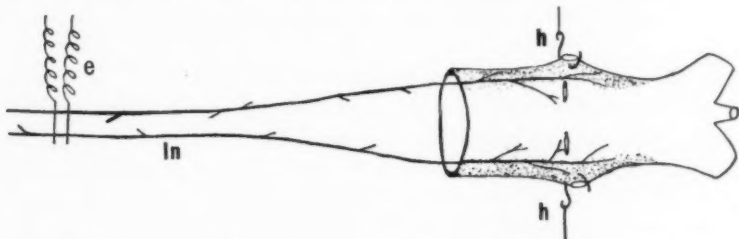


FIGURE 4.—Preparation of the lateral nerves and the first two heart-segments for studying the influence of the nerves on the resting muscle. Nerve-cord extirpated. *e*, electrodes; *h*, hooks for suspending the preparation for graphic registration; *ln*, lateral nerves.

lateral nerves. There is, however, in these experiments, no chance of escape of the current directly to the muscle, as the electrodes may be placed 5 to 6 cms. from the reacting segments.

Stimulation of both lateral nerves produces a greater contraction than when either nerve is stimulated singly. This is readily understood from the fact that each nerve is confined in its action almost wholly to its own side of the heart, the two nerves thus supplying separate portions of the musculature.

It is frequently not possible to augment the strength of the contraction by increasing the strength of the induced shock applied to the nerves above that which first proves effective, but such an augmentation in the amplitude of the contractions is always produced by two or more shocks following one another in rapid succession. Both these reactions are illustrated by the tracings in Figs. 5 and 6. In Fig. 5 the lateral nerve on the left side is being stimulated by break shocks of gradually increasing intensity. The numbers indicate the

position of the primary coil with reference to the secondary in centimetres. It is true that at 6, 5, and 4, the strength of the contractions is somewhat greater than at 7, but even with the secondary pushed clear over the primary (0), the contraction is not much greater than that produced by the minimal stimulus to the nerve. There is nothing that may be interpreted as an "all-or-nothing" response in these re-

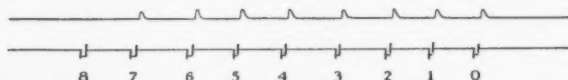


FIGURE 5.—Tracing of the contraction of the first segment on stimulation of the lateral nerves with single induced shocks. The strength of the shock is indicated by the figures which give the distance of the secondary from the primary coil in centimetres.

actions, for when small gradations in the strength of the induced shock are employed, corresponding slight gradations in the amplitude of the contractions may be observed; but the maximal response is quickly reached, beyond which no increase in the amplitude of the beat is produced by increasing the strength of the induced shock.

The accumulative effects of two or more induced shocks following one another at intervals of from one-eighth to one-fifth second is strikingly shown in Fig. 6. In this particular experiment both the

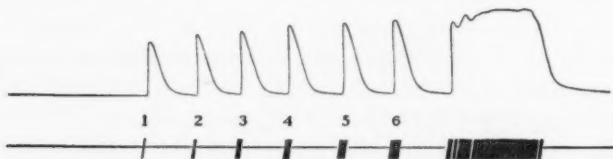


FIGURE 6.—Tracing from contraction of second and third segments. The lateral nerves stimulated with series of induced shocks. The figures give the number of shocks in the group, showing summation.

lateral nerves were placed on the electrodes. The figures give the number of shocks sent through the nerves. When the shocks are applied to the nerves at the rate of six to eight per second, an almost complete tetanus is produced. It is not clear whether this summation is to be referred to the nerve-fibres, so that a series of shocks in rapid succession produce a single strong nervous impulse, or whether the summation is in the muscle cells. The latter appears the more probable. That would imply that the heart-muscle of *Limulus* does

not conform to the "all-or-nothing" law, as usually interpreted for the vertebrate heart-muscle, and we shall see later that this is actually the case. The normal automatic beat of the *Limulus* heart is not maximal, as a stimulus applied at the beginning of systole may increase the strength of the contraction.

Stimulation of the lateral nerves with the interrupted current produces a continuous supermaximal contraction or tetanus of the heart-

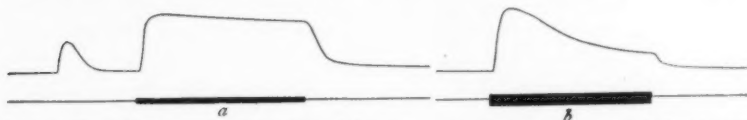


FIGURE 7.—Tracings of contraction of first segment. Lateral nerves stimulated with the interrupted current. *a*, from a fresh and vigorous preparation; *b*, from a fatigued preparation. Tetanus.

muscle. It is evident from the record in Fig. 6 that application of induced shocks to the nerves at the rate of eight to ten per second suffices to produce an incomplete tetanus, but in these experiments the automatic interrupter of the inductorium was usually employed. Fig. 7 *a* gives a typical curve of complete tetanus of the muscle of the first two segments on application of the interrupted current to the lateral nerves in a fresh and vigorous preparation. The particular character of the curve is the rapid attainment of the maximal degree



FIGURE 8.—Tracing from first segment on stimulation of lateral nerves with the interrupted current of increasing strength. Showing more complete tetanus with the stronger current.

of contraction, and the slow but steady relaxation that follows immediately upon the muscle's reaching this greatest degree of shortening. In this regard the form of the tetanus curve differs from that of the vertebrate skeletal muscle. When a preparation is fatigued, or in a poor condition, this immediate fall of the lever becomes more pronounced, as will be seen from Fig. 7 *b*. The relaxation also sets in sooner and proceeds more rapidly the weaker the strength of the interrupted current (Fig. 8). It is not clear whether the rapid fall of the curve is due to failure or fatigue of the muscle or of the nerve-fibres. It will probably be found that both factors are concerned.

When the nerves are being stimulated with a relatively weak interrupted current for some time, the tetanus curve usually exhibits the irregularities shown in Fig. 9. As the muscle relaxes, inco-ordinate or fibrillar contractions take the place of continuous contraction. This condition, not unlike the delirium of the vertebrate heart, is evidently only an incomplete tetanus, and may be due to fatigue of the muscle.

I have shown in a former paper¹ that stimulation of the cardio-accelerator nerves in molluscs produces contractions in the quiescent heart. To the various species of molluscs cited in that paper, in which this reaction may be observed, may now be added the large marine gasteropod *Sycotypus*. Stimulation of the nerve passing from the right visceral ganglion to a ganglion on the aortic end of the ventricle, or stimulation of the latter ganglion itself with the weak interrupted current, produces a series of beats or incomplete tetanus

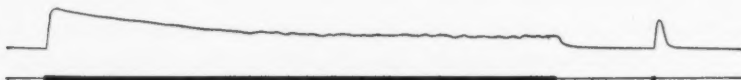


FIGURE 9.—Tracing from the first segment. Lateral nerves stimulated with a weak interrupted current. Tetanus curve passing into incomplete tetanus on long continued stimulation.

in the quiescent ventricle. But in reviewing the present status of the question whether the quiescent vertebrate heart can be made to beat by stimulation of the sympathetic nerves, I overlooked Stewart's observations² to the effect that the stimulation of the sympathetic produces contractions in the frog's heart brought to a standstill by raising the temperature of the heart. Stewart gives evidence to the effect that the heat standstill of the heart is not a vagus standstill. That the heart, including the sinus and the great veins, is really quiescent in the so-called heat standstill can readily be determined by aid of a lens. Actual motor effects, that is, the causing of a contraction in the quiescent heart-muscle on stimulation of any extrinsic or intrinsic cardiac nerves cannot, according to Engelmann, be admitted on the myogenic theory of the heart-beat,³ as that would imply

¹ CARLSON: This journal, 1904, xii, p. 55.

² STEWART: Journal of physiology, 1892, xiii, pp. 92, 93.

³ ENGELMANN: Das Herz und seine Thätigkeit im Lichte neuerer Forschung, Leipzig, 1903.

so great a modification of the theory as to virtually amount to abandoning it. Such true motor effects are, however, produced in the molluscan and the arthropod heart.

III. THE NERVE-CORD ON THE DORSAL SIDE OF THE HEART A REFLEX CENTRE.

Are the ganglion cells in the nerve-cord "automatic," or is their activity dependent on or influenced by impulses from intrinsic sensory nerves? The heart of *Limulus* lends itself admirably to the solution of this question. For studying the influence of possible afferent nerves on the ganglion cells, the heart is prepared in the manner shown in Fig. 10. One of the lateral nerves is carefully isolated from

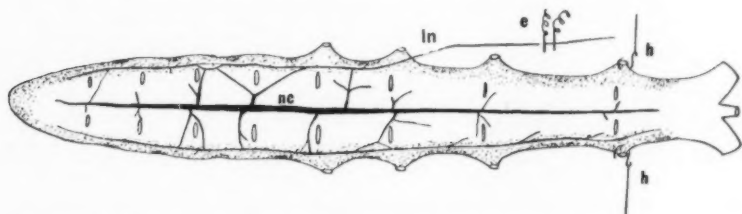


FIGURE 10.—Preparation of the heart for the study of the local cardio-reflex. Dorsal view. *nc*, nerve-cord; *ln*, lateral nerves; *h*, hooks for connecting a region of the heart with the lever; *e*, electrodes.

the muscle in the first three segments, and the isolated nerve stimulated. Any region or level of the heart may be connected with the recording lever, as this dissection of the nerve does not affect the rhythm or the co-ordination. The only effect is a diminution in the strength of contraction of the first three segments. If it is desired to study the effects of stimulation of both lateral nerves, it is best to connect the fifth or the sixth segment with the lever, as the dissection greatly diminishes the strength of the contraction in the anterior segments.

Now, when one or both lateral nerves prepared in this manner are stimulated with the weak interrupted current, the strength of the beats is increased, the rate of the beats may or may not be augmented, and in case the rhythm has ceased from exhaustion, a series of beats are produced during the stimulation. The augmentation of the amplitude of the beats is shown in the tracing reproduced in Fig. 11. In this particular experiment the tracing records the contraction of the

seventh segment; but the augmentation of the strength of the beats is evidenced in every part of the heart that remains in physiological connection with the nerve-cord. When the heart is beating vigor-

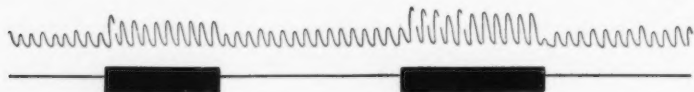


FIGURE 11. — Tracing from the first segment, showing reflex augmentation of the rhythm on stimulation of the lateral nerve in the manner shown in Fig. 10.

ously, the augmentation is usually less than shown in Fig. 11. The augmentation in the strength of the beat is usually accompanied by a quickened rate. In case the heart is beating very slowly, the augmentation of the rate may be very marked (Fig. 12).



FIGURE 12. — Tracing from sixth segment. Reflex augmentation of the rhythm on stimulation of the lateral nerves in the second segment. Heart beating slowly.

These effects of stimulating the lateral nerves in the anteroposterior direction are duplicated by stimulating any of the large

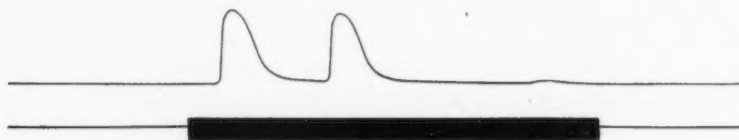


FIGURE 13. — Tracing from seventh segment. Lateral nerves stimulated in the second segment. Heart quiescent from exhaustion. Showing reflex contractions of quiescent heart.

branches leading from the nerve-cord to the lateral nerves in the middle region of the heart (Fig. 14 *a*). This was to be expected, as in this way we are stimulating the same neurones as in the case of the lateral nerves, only a little nearer the nerve-cord. When any one of these main connectives between the nerve-cord and the lateral

nerves is stimulated with a strong interrupted current, the very opposite effect, or inhibition of the rhythm is produced (Fig. 14*b*). I have reasons for thinking that this is not a true reflex inhibition, but due to the escape of the current directly to the ganglion cells in the nerve-cord. When these connectives are stimulated, the electrodes are at the most only 8 to 10 mm. from the cord, and as this inhibition is obtained only with very strong currents, escape of the current



FIGURE 14.—Tracing from second segment. Stimulation of one of the connectives between the cord and the lateral nerves in the sixth segment. *a*, weak current; *b*, very strong current. Showing opposite effects.

to the cord is very probable. A certain strength of the interrupted current applied directly to the nerve-cord produces inhibition. If there were any local inhibitory reflex mechanism in the heart, it ought to be revealed on stimulation of the lateral nerves 4 to 5 cms. distant from the cord; but when the position of the electrodes is removed that distance from the cord, even the greatest strength of the interrupted current produces only acceleration. Still, the possibility of the presence of a local inhibitory reflex mechanism must be admitted.

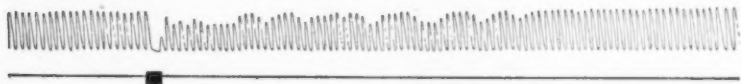


FIGURE 15.—Tracing from the first segment. Stimulation of one of the connectives between the cord and the right lateral nerve in the fifth segment. Strong interrupted current. Showing rhythmic variations in the strength of the beats.

The discussion of the nature of the inhibition produced by stimulating the cord directly, will be deferred to a later paper. The inhibitory effects produced by stimulating the connectives with a strong interrupted current persist much longer than the stimulation, and occasionally there appears a peculiar rhythmic variation in the strength of the beats before the original rhythm is resumed. A tracing showing these periodic variations is reproduced in Fig. 15. The base line remains horizontal, hence the variations in the excursions of the lever are not "tonus" contractions. They are actual variations in the

strength of the beat. Hence if one could have measured the blood-pressure in the aorta, one would have obtained typical "Traube-Hering-waves." The "Traube-Hering-waves" in the blood-pressure of mammals are ascribed to rhythmical variations in the size of the arterioles.¹ A periodic variation in the strength of the ventricular beat would produce the same effects on the arterial blood-pressure. These periodic variations in the strength of the beat in the heart of *Limulus* are due, not to any variations in the condition of the heart-muscle, but to variations in the activity of the nervous mechanism, — possibly the ganglion cells, as I have obtained tracings exhibiting these periodic variations after subjecting the nerve-cord to the action of certain solutions under conditions which absolutely prevented the solutions from coming in contact with the muscle of the region of the heart from which the records were taken.

The reflex contractions produced in the quiescent heart by stimulating the centripetal nerve-fibres in the lateral nerves are shown in the record reproduced in Fig. 13. In all the experiments I satisfied myself, by examining the heart with a lens, that it was actually quiescent in every segment. Only a few contractions, or at most a short series of contractions, follows the stimulation, even when the strength of the current is considerable. This is evidently due to the exhaustion of the nervous mechanism. But the contractions are sufficient in number to be recognized as a part of a true motor reflex.

These effects of stimulating the isolated lateral nerves of the first segment in the anteroposterior direction are also produced by stimulating the isolated lateral nerves of the last two segments in the posteroanterior direction. But as the lateral nerves are tiny and not so readily isolated in the eighth and ninth segments, nearly all the experiments were carried out on the lateral nerves of the anterior end of the heart. Sufficient work was, however, done on the nerves of the posterior end to prove that *afferent nerve-fibres pass to the nerve-cord from every region of the heart.*

What reasons are there for attributing these effects on the activity of the ganglion cells to stimulation of centripetal or sensory nerves? May they not be simply "axon reflexes," in Langley's sense? The following facts speak against "axon reflexes." The change in the rhythm involves every part of the heart-muscle not severed from its connection with the nerve-cord. This is the case whether one or

¹ WOLF and PLUMIER: *Journal de physiologie et pathologie générale*, 1904, vi, p. 213.

both lateral nerves are stimulated, as well as when the electrodes are applied to any one of the main connecting branches between the nerve-cord and the lateral nerves. On the "axon reflex" hypothesis, every motor neurone in the nerve-cord would thus have to send axis cylinder processes to practically every muscle cell in the heart, which is highly improbable, if not impossible. The principle of reciprocal conduction in nerve-fibres does not suffice as an explanation of the augmented rhythm. It appears to me fairly well established in the case of the vertebrates, that the nervous impulses that pass centripetally in the motor nerve-fibres do not produce any effect on the nerve-centres. Sensation or motor responses cannot be elicited in that way. There is no reason for supposing that the physiological polarity of the conducting elements in the nerve-centres is less marked in *Limulus* than in the vertebrates. We must, therefore, fall back on the most natural explanation, viz., that the change in the rhythm on stimulation of the lateral nerves is a true reflex. The nerve-cord is the reflex centre. Sensory nerves pass from the walls of the heart to the nerve-cord. The existence of this local cardiac reflex mechanism appears to me sufficient evidence of its being functional in the normal heart activity, but whether the rhythmical activity of the ganglion cells is actually caused by or merely influenced by these centripetal nervous impulses cannot be answered without further study.

That the rhythm of the vertebrate heart can be influenced reflexly by stimulation of sensory nerve-endings or nerves in the heart has been shown by Muskens.¹ But according to this observer, the centre for these reflexes is in the central nervous system and not in the ganglia in the heart itself. That the ganglia in the vertebrate heart function as reflex centres has, to my knowledge, not yet been demonstrated. It is of interest to note that the reflex effects observed by Muskens on the frog's heart were inhibitory, while the local reflexes in the *Limulus* heart appear to be solely motor or accelerator. I expect that the local reflexes in the vertebrate heart will also prove to be motor or accelerator.

IV. THE EFFECT OF TENSION ON THE HEART WALLS.

It is well known that in vertebrates tension on the heart walls up to a certain limit is favorable to the rhythm, and may thus be said to act

¹ MUSKENS: *Archiv für die gesammte Physiologie*, 1897, lxvi, p. 328.

as a stimulus. The beats become stronger, and the rate may be augmented. Tension may also cause a beat or a series of beats in the quiescent heart. Thus it is said that by raising the pressure of the blood in the ventricle the apex of the frog's ventricle rendered quiescent by Bernstein's crushing is made to beat rhythmically. Gaskell believes that mechanical tension may cause a rhythm in isolated strips from the apex of the tortoise's ventricle. I have at times obtained a series of beats of the intact but quiescent ventricle of the dog simply by stretching the ventricular wall. This stimulating effect of tension on the heart walls is very manifest in the invertebrates, as shown by the works of Foster and Dew-Smith, Biedermann, Ransom, Schoenlein, and Straub.¹ These observers worked on the hearts of various molluscs, all obtaining substantially the same results. These results I have confirmed on the hearts of several molluscs. Up to a certain limit, the augmentation of the rhythm is directly proportional to the pressure of the fluid in the cavity of the heart. If the pressure is rapidly increased, the heart may go into a condition of tonus or fibrillary contractions. Simple mechanical tension exerted on the heart wall is on the whole less efficient as a stimulus to the rhythm than the pressure exerted by a liquid in the heart cavity. Yet in some molluscs (for example, the giant California slug *Ariolimax*), there is little difference between the results of these two ways of producing tension in the heart walls. The excised and therefore empty and collapsed ventricle of the slug (*Ariolimax*) beats with perfect rhythm for hours. If the ventricle is suspended and loaded with a light recording lever, the rhythm becomes more rapid and vigorous. The stimulating action of the tension from the load is shown still more clearly when the empty heart has been beating for six to eight hours, and is becoming exhausted. With the ventricle in an exhausted condition, releasing the tension stops the rhythm, and reapplying the tension is followed by the reappearance of the rhythm. *Simple mechanical tension on the ventricular walls is thus able to produce a rhythm in the quiescent ventricle.* But this does not prove that the rhythm is produced by the tension acting on the muscle.

The heart of arthropods is not so well adapted to studying the

¹ FOSTER and DEW-SMITH: Proceedings of the Royal Society, 1875, xxiii, p. 318; BIEDERMANN: Sitzungsberichte der Wiener Akademie, 1884, lxxxix, 3, p. 191; RANSOM: Journal of physiology, 1884, v, p. 261; SCHOENLEIN: Zeitschrift für Biologie, 1894, xxx, p. 187; STRAUB: Archiv für die gesammte Physiologie, 1901, lxxxvi, p. 504.

effects on the rhythm of varying the pressure of the liquid in the heart cavity, because of the several ostia opening into and the several arteries leading from the heart. In the *Limulus* heart the pressure in the cavity may be raised by ligaturing the lateral arteries and admitting plasma or sea-water by a cannula at the aortic end. The pressure of the liquid may be varied by varying the inclination of the cannula. The *Limulus* heart responds to variations in pressure in every way like the heart of vertebrates and molluscs. Increasing the pressure up to a certain limit, augments the rhythm, a further sudden increase causes inco-ordination, fibrillary contractions, and incomplete tetanus or tonus, just as Biedermann observed in the ventricle of the snail. If the *Limulus* heart is quiescent from exhaustion, the mere filling of the heart with plasma or sea-water under slight pressure may start a series of beats. That the tension or pressure of the liquid on the heart walls, and not any chemical action, is the stimulating factor seems to be shown by the fact that immersion of the whole heart in the plasma or sea-water does not produce similar results. Augmentation of the rhythm can also be produced by simple mechanical tension on the heart. If the suspensory ligaments attached to either lateral angle of the heart in the fifth or sixth segments are gently stretched, and the heart thus put under tension, the rhythm of the whole heart is augmented, despite the fact that the tension affects at the most only two segments. The same results may be obtained by inserting hooks at opposite angles of the heart in the middle region, securing the one to a fixed support, and to the other one attaching weights of different values. When mechanical tension is thus applied in the middle region, I have even succeeded in starting a series of beats in perfectly quiescent hearts, that is, in hearts quiescent from exhaustion. The mechanical tension is most efficient in the middle region of the heart. When the first two or the last two segments are similarly stretched, the augmentation, if produced, may be confined to the region of the tension, thus resulting in inco-ordination of the rhythm.

The pressure of the liquid in the heart cavity and the simple stretching of the heart walls produce these effects only in hearts with the ganglion or nerve-cord intact. After extirpation of the nerve-cord I have never succeeded in producing a beat or a series of beats by these means. When the nerve-cord is extirpated, the heart becomes quiescent. Now if hydrostatic pressure or mechanical tension acted as stimuli only on the beating heart, we would not get far in our

analysis of the nature of these stimuli, but inasmuch as they produce a series of beats in the quiescent heart as long as the ganglion is intact, but not after extirpation of the ganglion, it is evident at once that their action is in some way dependent upon the nerve-cord. In the first place, tension does not produce a rhythm by direct action on the heart-muscle or on the motor nerves and nerve-endings. We know, however, that the stretching of a nerve or nerve-fibre up to a certain limit increases its excitability. It is therefore not impossible that the tension on the heart walls increases the excitability of the motor fibres so that the nervous impulses reaching the heart-muscle from the ganglion cells become of greater strength. That may suffice to explain the augmentation of the amplitude of the beats, but it does not account for the increased rate. That must be due either to direct action of the tension on the ganglion cells, or to stimulation of sensory nerve-endings and fibres in the walls of the heart, making it a true reflex action. We have shown that such a local reflex mechanism exists in the heart of *Limulus*, and it is not improbable that these sensory nerves are readily stimulated by tension. The tension or pressure may furthermore act directly on the ganglion cells.

Theoretically it ought to be possible to produce a rhythm in the heart of *Limulus* by simple tension of the heart walls, even after extirpation of the nerve-cord. By stretching a motor nerve up to a certain limit, we can produce a contraction or a series of contractions in the muscle supplied by the nerve. Now if stretching the heart walls put sufficient tension on the motor nerve-fibres to start a nervous impulse, the contraction of the muscle would immediately release the tension on the nerves, while the subsequent relaxation of the muscle would again allow the full strength of the tension to act on the nerves, resulting in a second stimulus of the nerve-fibres and a second contraction of the heart-muscle, etc. But the motor nerve-fibres in the heart of *Limulus* are evidently sufficiently elastic not to be stimulated by a moderate amount of stretching of the heart walls. There may, however, be hearts having sufficiently inelastic and excitable motor nerves for tension to produce rhythmical contractions in this manner in the absence of ganglion cells or a reflex nerve-centre. The stimulating action of tension in the vertebrate heart is usually interpreted as being on the heart-muscle directly. In view of these results on the *Limulus* heart, it would seem that the question should be re-examined in the vertebrates.

V. THE INFLUENCE OF SODIUM CHLORIDE ON THE HEART, WITH
AND WITHOUT THE NERVE-CORD.

When a *Limulus* heart is removed from the plasma and placed in an isotonic ($\frac{5}{8}m$) sodium chloride solution, the rhythm is at once augmented. The rate of the beats is particularly increased. A heart that is quiescent from exhaustion starts to beat within one to three minutes after being placed in an isotonic sodium chloride solution. It does not concern us for the present how long this rhythm is maintained. It suffices to note that an isotonic sodium chloride solution augments the rhythm of the beating heart at once or after a latent period of one to two seconds, and starts a series of beats in the quiescent heart after a latent period of not exceeding three or four minutes. Now when the heart is placed in the sodium chloride solution after extirpation of the ganglion, it remains perfectly quiescent for thirty to forty-five minutes. The same is true whether the whole heart is immersed in the solution or only a portion of the heart wall is used. There is, then, this difference in the action of the sodium chloride on intact and on the ganglion-free heart; viz., in the first case, the solution stimulates at once, in the latter case, it also stimulates or causes a rhythm of the heart-muscle, but only after a latent period of more than thirty minutes. The action of the solution on the intact heart is therefore an action on the ganglion, or a local reflex dependent on the integrity of the ganglion. The belated action on the heart deprived of the nerve-cord must be an action on the motor nerve-fibres or on the muscle cells directly. It is only in a pure sodium chloride solution that this rhythm is developed in a heart from which the nerve-cord has been removed. The rhythm does not appear when the heart is left in plasma, or sea-water, or in an artificial solution containing the chlorides of sodium, calcium, and potassium in approximately the same proportions as the sea-water, while the heart with the nerve-cord intact beats rhythmically in either of these solutions, as well as in the air, when protected from evaporation. The ganglion-free heart of *Limulus* thus reacts to a solution of pure sodium chloride in the same manner as do vertebrate skeletal muscle or the apex of the frog's and tortoise's ventricle. For notwithstanding the observations of Gaskell, it has recently been asserted¹ that the ventricular tissue of the tortoise's heart is not spontaneously rhythmical under the conditions of normal life.

¹ MARTIN: This journal, 1904, xi, p. 103.

The nature of the action of sodium chloride on muscle in producing rhythmical contractions is not yet understood, but this action of the salt is frequently cited as a proof of the myogenic theory of the heart-beat. That inference is entirely unwarranted, for the combination of salts in the blood does not stimulate muscle or nervous elements in this way, and no tissue is bathed in a pure sodium chloride solution under normal conditions.

VI. DIRECT STIMULATION OF THE HEART.

It has been claimed that the heart of cephalopods and tunicates exhibits a refractory condition during systole similar to that of the heart of the higher vertebrates.¹ This is not true if we understand by refractory period a condition of inexcitability. The excitability of the arthropod, the molluscan, and the tunicate heart is lowest at the beginning of systole, but a strength of the induced shock can be found which affects the heart in any phase of contraction.² The heart of *Limulus* makes no exception to this rule. An induced shock of sufficient strength sent through the heart at the beginning of systole produces a supermaximal beat. The so-called "all-or-nothing" law is, furthermore, not applicable to the invertebrate heart. I have recently shown that the "all-or-nothing" principle and the principle of absolute inexcitability during systole does not apply to the heart of the lowest vertebrates.³ It is true that the heart, especially that of cephalopods and crustaceans, when in good condition, tends to respond with a beat of uniform strength to stimuli of varying intensity within a wide range, but increase in the strength of stimulus above this range produces supermaximal contractions. The heart of pulmonates and the heart of *Limulus* exhibits perhaps the least of this tendency to a response of uniform strength.

A Limulus heart with the nerve-cord intact exhibits a much greater excitability and a greater tendency to uniform contractions in response to stimuli of varying strengths than does the same heart after the nerve-cord has been extirpated. There is the further difference that a single induced shock, however strong, sent through a ganglion-free heart produces only a single contraction, while the same shock applied to a quiescent but intact heart may produce a series of beats.

¹ RANSOM: Journal of physiology, 1884, v, p. 261; SCHULTZE: Jenaische Zeitschrift für Naturwissenschaft, 1901, xxxv, p. 221.

² CARLSON: Science, 1903, xvii, p. 548.

³ CARLSON: Zeitschrift für allgemeine Physiologie, 1904, iv, p. 259.

The single induced shock is evidently not able to produce a series of beats when acting on the motor nerve-fibres and the muscle alone, while a single stimulation of the ganglion cells may cause a rhythmical activity of the latter. The greater tendency of the heart to uniform response when the ganglion is left intact, is illustrated by the tracings

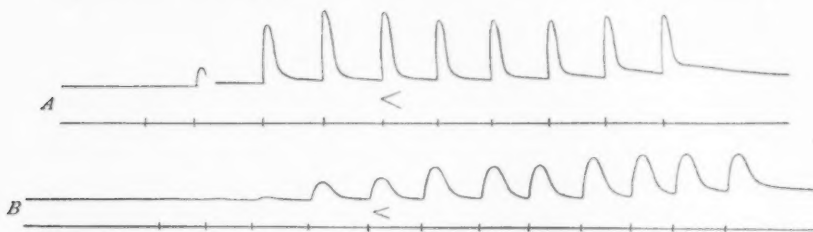


FIGURE 16.—Contractions in response to direct stimulation of the heart with single-induced shocks of increasing strength. *A*, from the third segment, with the nerve-cord intact; *B*, from the sixth segment, with the nerve-cord extirpated. Showing tendency to greater uniformity in the contractions when the cord is intact.

in Fig. 16. The upper record is from the contraction of the third segment in an intact heart, the latter from the contraction of the sixth segment in a ganglion-free heart. In both cases the induced shocks are sent through the heart from side to side in the segment connected with the recording lever.

It is needless to add that the heart of *Limulus* can be tetanized by direct stimulation with a strong interrupted current, as that follows



FIGURE 17.—Tracing from first segment. At *X* the nerve-cord was touched with the forceps in the fifth segment. Showing acceleration of the rhythm on mechanical stimulation of the ganglion.

necessarily from the fact that the muscle can be tetanized by stimulation of the motor nerves.

The difference in the response of the heart to direct stimulation, according as the ganglion is intact or extirpated, goes to show that at least a part of the peculiarity of the properties of cardiac tissue in response to direct stimulation is due to the presence of nerve-centres or ganglion cells, and a relatively low excitability of the motor nerve-fibres and the muscle to the induced current. It is scarcely neces-

sary to point out that we do not yet know the properties of the heart-muscle apart from the intrinsic heart-nerves, as we know of no drug that will paralyze the motor nerve-endings in the heart without injury to the heart-muscle.¹

Since the recent (1902) comprehensive review of the literature on the vertebrate heart with special reference to the neurogenic or the myogenic nature of the heart-beat, and the co-ordination in the heart by Langendorff,² no contributions to vertebrate heart physiology of decisive bearing on these questions have been made. But it appears to me not out of place in this connection to call attention to some erroneous statements in current literature regarding the presence of nervous elements in the heart of invertebrates. Thus Langendorff says in his otherwise able review: "It has been determined by numerous researches that in the case of the heart of many invertebrates (molluscs, arthropods, tunicates, crustaceans), the presence of ganglion cells and nerve-fibres cannot be demonstrated (pp. 334, 339). In his recent (1903) address on the physiology of the heart, Engelmann gives expression to the same view: "In the heart of higher invertebrates (tunicates, molluscs, arthropods) Foster and his students were unable to find ganglion cells, and the painstaking researches of later investigators (Biedermann, Knoll, Straub, Schultze, Heine) have led to the same results" (p. 10). Practically the same statement is found in many textbooks of physiology. Porter puts it in this way: "The hearts of many invertebrates in which ganglion cells are apparently absent beat rhythmically."³

Turning our attention first to the molluscs, we find that Foster,⁴ Foster and Dew-Smith,⁵ Darwin,⁶ and Biedermann⁷ claim that there are neither nerve-cells nor nerve-fibres in the heart of the snail. Darwin cautions us against placing too much value on his negative results. Biedermann had recourse only to the method of macerating the heart in caustic potash and teasing. Results similar to these

¹ HERING, H. E.: *Archiv für die gesammte Physiologie*, 1903, xcix, p. 253; CARLSON: *Science*, 1904, xx, p. 684.

² LANGENDORFF: *Ergebnisse der Physiologie*, 1902, i, pp. 263, 345.

³ PORTER: *American textbook of physiology*, 1900, i, p. 151.

⁴ FOSTER: *Archiv für die gesammte Physiologie*, 1875, v, p. 191.

⁵ FOSTER and DEW-SMITH: *Proceedings of the Royal Society*, 1875, xxiii, p. 318; *Archiv für mikroskopische Anatomie*, 1877, xiv, p. 317.

⁶ DARWIN: *Journal of anatomy and physiology*, 1876, x, p. 506.

⁷ BIEDERMANN: *Sitzungsberichte der Wiener Akademie*, 1884, lxxxix, 3, p. 19.

were obtained by Knoll¹ on the heart of some heteropods. This investigator was equally unable, making use of the gold chloride method, to find nerves or ganglion cells in the heart. These observations are certainly erroneous as regards the presence of *nerve-fibres* in the heart, as shown by the works of Ransom,² Young,³ Dogiel,⁴ Bottazzi and Enriques,⁵ and Budington.⁶ Ransom has shown both by histological and physiological methods that the snail heart is provided with nerves. Young and Budington have proved that the heart of several lamellibranchs is supplied with nerves from the visceral ganglion. The same has been shown histologically by Dogiel. Ransom and Bottazzi and Enriques have further demonstrated that the heart of *Aplysia* is similarly provided with nerves. That the heart of the cephalopods is provided with nerves has been known since the observations of Paul Bert on *Sepia*, more than thirty years ago. To these observations showing the presence of nerves in the heart of various molluscs may be added my own (the details of which have not yet been published), made on several representatives from every group in the phylum, and they are to the effect that cardio-regulative nerves, accelerator or inhibitory, or both, are uniformly present. This makes it highly probable that *the molluscan heart is without exception provided with cardio-regulative nerves.*

Regarding the presence of *nerve-cells* in the molluscan heart, it is true that Foster, Foster and Dew-Smith, Darwin, Biedermann, Ransom, and Knoll failed to find any, but these observers (excepting Ransom) also failed to find nerve-fibres in the heart, although the nerve-fibres are certainly there. Their methods were therefore at fault, and it is probably just as erroneous to conclude from their observations that no ganglion cells are present in the heart, as it would be to conclude that cardio-regulative nerves are absent. We have, moreover, positive evidence of the presence of ganglion cells in the molluscan heart. Dogiel (*loc. cit.*) described and figured nerve cells in the auricles and the ventricles of *Helix*, *Pecten*, *Anadonta*, and *Aplysia*. Haller⁷ describes and figures both multipolar

¹ KNOLL: Sitzungsberichte der Wiener Akademie, 1893, cii, 3, p. 387.

² RANSOM: Journal of physiology, 1884, v, p. 261.

³ YOUNG: Archives de zoologie expérimentale, 1881, ix, p. 429.

⁴ DOGIEL: Archiv für mikroskopische Anatomie, 1877, xiv, p. 59.

⁵ BOTTAZZI and ENRIQUES: Archives italiennes de biologie, 1901, xxxv, p. 111.

⁶ BUDINGTON: Biological bulletin, 1904, vi, p. 311.

⁷ HALLER: Zoologisches Jahrbuch, 1883, ix, p. 1.

and bipolar ganglion cells in the heart of *Fisurella* and the *Muricidae*, and he traces the processes from the cells to nerve plexuses about the muscle-fibres. The same author describes under the ectocardium of the heart of Chitons a plexus of ganglion cells and nerve-fibres very similar to that in the sinus and the auricles of the vertebrates.¹ More than fifty years ago Hancock and Embleton² described a ganglion on the aortic end of the heart of *Doris*. This I have confirmed on a related species of the Pacific coast. In the marine gasteropod *Sycotypus*, I found a similar ganglion at the ventriculoaortic junction. I am not familiar with the work of Heine, referred to by Engelmann in the statement cited, but Engelmann, citing Straub's work as showing the absence of nervous elements in the heart, is certainly erroneous. For I take it that Engelmann refers to Straub's work on the heart of *Aplysia*.³ In that paper Straub *assumes* the absence of nervous elements in the heart, but he brings no proofs, either histological or physiological, in support of the assumption, and he furthermore appears to have been ignorant of the observations of Dogiel and Ransom proving the presence of cardio-accelerator nerves to the *Aplysia* heart.

In the case of the Arthropod heart there can be no question of the presence of cardio-regulative nerves from the thoracic ganglion, at least in the crustaceans,⁴ Knoll's negative results notwithstanding. I have confirmed the earlier observers both on *Macrura* and *Brachyura*. The papers of Milne-Edwards,⁵ and Patten and Redenbaugh⁶ leave no doubt as to the presence of *nerves* in the heart of Arachnids. The presence of *ganglion cells* in the heart of the Arachnids (*Limulus*) is also conclusively proven by Patten and Redenbaugh, and they are demonstrated with such comparative ease that any one in doubt as to the accuracy of the observations of Patten and Redenbaugh may readily convince himself of the presence of ganglion cells in the nerve-cord and of their true nervous nature. The evidence for

¹ HALLER: Arbeiten aus dem Zoologischen Institut der Universität in Wien, 1882, iv; 1883, v.

² HANCOCK and EMBLETON: Philosophical transactions, 1852, Part I, p. 207.

³ STRAUB: Archiv für die gesammte Physiologie, 1901, lxxxvi, p. 504.

⁴ PLATEAU: Bulletin de l'académie royal de Belgique, 1878, xlv; Archives de biologie, 1880, i, p. 595; YOUNG: Archives des zoologie expérimentale, 1878, vii, p. 401; JOLYET and VIALLANES: Annales des sciences naturelles, zoologie, 1892, xiv, p. 387; CONANT and CLARKE: Journal of experimental medicine, 1896, i, p. 341; BOTTAZZI: Centralblatt für Physiologie, 1901, civ, p. 663.

⁵ MILNE-EDWARDS: Annales des sciences naturelles, 1873, xvii, ser. 5.

⁶ PATTEN and REDENBAUGH: Journal of morphology, 1899, xvi, p. 91.

the presence of ganglion cells in the crustacean heart is no less convincing. It is true that some of the early observers failed to find nerve-cells in the heart of crabs and crayfishes, but the positive results of Berger,¹ Dogiel,² Deszö,³ and Pogoschewa⁴ must take precedence over the earlier negative findings. It is inconceivable that the elements figured and described by Dogiel in one of his late papers on the crustacean heart (1894) can be anything but nerve-cells. Dogiel has, furthermore, described nerve-cells in the heart of the *Corethra larva*.⁵

The tunicate heart was the last to give up its secrets in that nervous elements in it were sought for in vain up till very recently. The reader will find the references to the earlier observations in the recent paper by Schultze.⁶ Schultze was equally unable to find any nerves or nerve-cells in the heart of *Ciona* or *Salpa*. But all these failures, it appears to me, do not count against the clear demonstration of nerve-cells and nerve-fibres in the heart of *Molgula* by Hunter.⁷ It is significant that in the tunicate heart the ganglion cells are massed particularly at either end, that is, in the two regions where the reversible rhythm originates.

Nerves and nerve-cells have thus been shown to be present in the heart of a great number of molluscs and arthropods, and at least in one tunicate. There can be little doubt but that with greater accuracy in histological and physiological methods and observations, nervous elements will be found to be present in the heart of all invertebrates. But even on the basis of the data so far at hand, it is obvious that statements like those cited on page 504 are wholly erroneous.

SUMMARY.

The ganglion cells of the venous end (fifth, sixth, and seventh segments) of the heart of *Limulus* are more numerous and exhibit greater automatism than the ganglion cells of the aortic or anterior end. From the ganglion cells in the fifth, sixth, and seventh seg-

¹ BERGER: Sitzungsberichte der Wiener Akademie, 1876, lxxiv, Abt. i, p. 422.

² DOGIEL: Archives de physiologie, 1877; Archiv für mikroskopische Anatomie, 1894, xliii, p. 223.

³ DESZÖ: Zoologischer Anzeiger, 1878, i, p. 126.

⁴ POGOSCHEWA: Bote für Naturwissenschaften, 1890, No. 5.

⁵ DOGIEL: Mémoires de l'académie St. Petersburg, 1877, xxiv, No. 10.

⁶ SCHULTZE: Jenaische Zeitschrift für Naturwissenschaften, 1901, xxxv, p. 221.

⁷ HUNTER: Anatomischer Anzeiger, 1902, xxi, p. 241; Science, 1903, xvii, No. 424; This journal, 1903, x, p. 1.

ments nerve-fibres pass in the nerve-cord and the lateral nerves directly to the heart-muscle of every segment.

The nerve-fibres passing from the ganglion cells in the nerve-cord to the heart-muscle are ordinary motor fibres. They exhibit a very low excitability to the induced current; nevertheless the heart-muscle responds to single induced shocks applied to the nerves. Stimulation of the nerves with the interrupted current produces tetanus of the heart-muscle.

The ganglion or nerve-cord on the dorsal side of the heart is a reflex centre. Stimulation of the sensory or centripetal nerve-fibres passing from the walls of the heart to the nerve-cord augments the rhythm, and may start a rhythm in a heart that is quiescent from exhaustion. There is no evidence of the presence of a local inhibitory reflex mechanism.

On direct stimulation of the heart, the amplitude of the contraction varies, within limits, directly with the strength of the stimulus; but there is a greater tendency to uniform contractions in response to stimuli of varying intensity in hearts with the nerve-cord intact, than in hearts from which the nerve-cord has been removed.

A certain degree of mechanical tension on the heart walls, or pressure of an indifferent liquid in the cavity of the heart, augments the rhythm, and may start a rhythm in hearts that are quiescent from exhaustion. These effects are produced only in case the nerve-cord is intact. After extirpation of the ganglion, neither hydrostatic pressure in the heart cavity, nor mechanical tension on the heart walls, produces contractions.

The action of inorganic salts, particularly sodium chloride, in solution on the ganglion appears to be of the same nature as that on the muscle, except that they act more rapidly on the ganglion cells. An isotonic sodium chloride solution stimulates the ganglion practically instantaneously, while the heart from which the nerve-cord has been removed develops a more or less rhythmical series of contractions only after a prolonged (thirty to forty-five minutes) immersion in the solution. Calcium chloride counteracts the stimulating effects of the sodium chloride both on the ganglion and on the muscle. The combination of salts in the blood or in the sea-water does not produce contractions of the heart after the nerve-cord has been removed.

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